On biogeochemical processes influencing eutrophication and toxicity in riverine wetlands

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Vormgeving:
Rob van de Haterd & Roos Loeb

Druk:
Gildeprint Drukkerijen BV, Enschede

Beeldmateriaal:
foto voorzijde (overstroming): Jouke Altenburg
foto voorzijde (droge situatie), p165, p166: Rob van de Haterd
luchtfoto achtergrond p8 e.v., p170, p172: Leon Lamers
tekeningen p137,139,141: Roos Loeb & Rob van de Haterd
dijkdoorbraak p156: Atlas van Stolk, Rotterdam
overige foto's Roos Loeb

On biogeochemical processes influencing eutrophication and toxicity in riverine wetlands

Een wetenschappelijke proeve op het gebied van de
Natuurwetenschappen, Wiskunde en Informatica

Proefschrift

ter verkrijging van de graad van doctor
aan de Radboud Universiteit Nijmegen
op gezag van rector magnificus prof. mr. S.C.J.J. Kortmann,
volgens besluit van het Collega van Decanen
in het openbaar te verdedigen
op woensdag 5 november 2008
om 13:30 precies

door

Rozemarijn Loeb

geboren op 24 april 1977
te Renkum

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Manuscriptcommissie:
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Paranimfen:
Marlies van der Welle
Jeroen Geurts

ISBN: 978-90-9023545-5

Laag hangt de zon. De lange bosschen, dijken
Van ondoorschijnendheid, weren de baren
Van 't roode licht, dat afdruipt van de blaren,
Doorsijp'lend, waar tot lek de twijgen wijken;

De vlakke stroomen, die 't doorzichtig strijken,
Kan niet het voorland, ruigbegroeid met varen,
Niet kan de takkenglooiing doen bedaren
De steile vloed, die heen spoelt over de eiken;

Over de kruin en –dijkbreuk– door de wanden
Stort zich de oranje branding op de landen,
Wijd vloeiend goud, als uit een fabelbron;

In 't oosten bouwt de nacht zijn wolkendammen;
Meezuigt de zon de vloed van koele vlammen,
En ze ebben weg onder de horizon.

JOHAN ANDREAS DÈR MOUW

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1

General introduction

Introduction

River ecosystems are among the most impacted ecosystems around the world (Tockner & Stanford, 2002). In the northern part of the world as much as 77% of the rivers are affected by fragmentation and flow regulation (Dynesius & Nilsson, 1994). Unimpacted rivers in this region are almost only found in Scandinavia and in that region only the smaller rivers amongst them (Dynesius & Nilsson, 1994). Physical changes such as flow regulations have severely impacted river hydrology, and flood regulation through endikement has rectricted the influence of the rivers from floodplains of tens kilometers wide to narrow floodplains of a few hundreds of meters to a few kilometers most of the times (Van Urk & Smit, 1989). Apart from the physical changes, also river water quality changed. Rivers are used as 'open sewers' which effectively carry industrial, agricultural and domestic waste to the sea. Additionally, land-use changed and many floodplains are used as agricultural land, implicating nowadays the use of fertilisers and pesticides. These chemical changes have impacted river ecosystems along anthropogenically modified rivers. This thesis focusses on the effects of these chemical alterations that took place and are taking place in river systems in the Netherlands. Notwithstanding the local focus, the processes described in this thesis are applicable for impacted river systems in temperate regions throughout world.

Historical changes in the Dutch river delta

The riverine area is one of earliest inhabited landscapes in the Netherlands. The first settlements were built on the levees of the large rivers. Here, the inhabitants found a combination of fertile floodplains, a sufficient water supply and possibilities for transportation, as the accessibility of the woody and swampy hinterland was poor. Earliest settlements date back to 1800-800 B.C. (Wolf *et al.*, 2001). In the beginning of the inhabitation period, settlers endured frequent floods and shifts of the river beds, but starting from the Early Middle Ages dikes were built. The first dikes were placed around the towns, protecting the towns themselves, but not the neighbouring lands (Wolf *et al.*, 2001), keeping intact the floodplain gradients with the sandy levees and the lower, wet backswamps. Later on, the rivers were diked, fixing the river beds and decreasing the area subjected to floods to the area in between the dikes ('uiterwaarden' in Dutch). From 1450 on, the major rivers in most areas in the Netherlands were diked and in the 19th century river channels were straightened and fixed (Van Urk & Smit, 1989). As a result of these measures, water levels during peak discharge became higher, because the surplus of water was distributed over a smaller area. Sedimentation of sand and clay became limited to the area in between the river dikes, raising the remaining floodplains in relation to the land behind the dikes, increasing high water levels even more. This caused a change in the yearly water table fluctuation in the river Rhine from 1.5 -2.5 m a thousand years ago to 7 - 10 m nowadays (Nienhuis *et al.*, 2002).

Because of the early influence of men on the riverine landscape, a clear prehistorical reference of the area is not available. It is however likely that the area neighbouring the large rivers was covered with vast willow (*Salix*) woods and woods consisting of oak (*Quercus*), ash (*Fraxinus*) and elm (*Ulmus*) trees on higher elevated places (Wolf *et al.*, 2001). The high river dynamics offered spots for herbs, at places where trees were unable to grow. River bed shifts left wet places behind, such as oxbow lakes. These places offered space to less dynamic nature, such as to alder (*Alnus*) carrs and to the formation of peat fens.

The riverine forests were cut down for wood or to take the fertile land in production, and trees are nowadays still cut to avoid obstruction of river water at high discharge (Nienhuis & Leuven, 2001). The new agricultural land was used as pastures, as cropland -usually protected by extra dikes preventing summer floods- and as hay meadows. As the low laying backswamps ("komgronden") stayed wet the largest part of the year, they could only be used for the cultivation of willows for a long time. For this reason, they were the latest (as late as the 1950s) to be drained and transformed into pastures and cropland (Brusse, 2002). Besides the agriculture, also brick factories and inland shipping became

of major economical importance in the riverine area. Brick factories mined sedimentated clay in the floodplains (Jongman, 1992), leaving patterns of new 'lakes' behind. Inland waterway shipping forms approximately 17% of the freight traffic in the Netherlands (Bus, 2007). It demands deep enough river beds to navigate through.

The development in the estuaries has been a different one. Here, flooding occurred twice a day. Only a small part of the estuaries was influenced by brackish water. The largest part consisted of fresh water tidal area, where the tide was caused by the propulsion of fresh river water by the tides at sea. In the brackish water tidal area plants, such as *Cochlearia officinalis*, were adapted to haline conditions. In the freshwater tidal area no such adaptations were needed, but the presence of a tidal amplitude still led to the development of characteristic plant communities. Willow woods vegetated with the rare species *Caltha palustris* subsp. *araneosa* and *Leucojum aestivum* are characteristic for forests in this area (Wolf *et al.*, 2001). Sites lower in elevation were mainly vegetated by tall helophytes such as reed (*Phragmitis australis*) and rushes (*Schoenoplectus* and *Bulboschoenus* species). Here, people mainly used the original vegetation for a living. Willows were cultivated for making baskets and dikes (Thijsse, 1938; Burm & Haartsen, 2003) and rushes were cultivated for the production of furniture. Not until the 20th century, radical changes occurred in the estuaries. In 1932 the Zuiderzee was closed off and now forms the freshwater Lake IJsselmeer, removing the estuarine fresh-haline gradient from the river IJssel. In 1953 a major storm surge caused a flood catastrophe in the south-western part of the Netherlands, in which 1835 people were killed (Smits *et al.*, 2006). The brackish water also caused large damage for agriculture in this area. To prevent such catastrophes in future, the Delta Plan was drawn up. In this plan it was decided to close-off the Rhine-Meuse estuary almost entirely. In 1971 the Haringvliet was closed off from the sea as a part of this plan. The Haringvliet sluices now empty the river water into the sea during low tide, but are closed during high tide, preventing the intrusion of salt water. The Haringvliet therefore changed from a brackish tidal area into a stagnant, freshwater lake. The only open waterway connected to the sea is now the Nieuwe Waterweg, enabling containerships to reach the Rotterdam harbour. Along the Nieuwe Waterweg, the only remaining brackish tidal area is found, and the river Oude Maas, flowing into the Nieuwe Waterweg, is the only real remainig freshwater tidal river in the Netherlands. Through this river, tidal pulses still reach the freshwater tidal nature reserve the Biesbosch, in which the tidal amplitude changed from 2 to 0.3 m after the closure of the Haringvliet. In this nature reserve the intertidal area decreased from 3750 ha before the closure to 120 ha today due to the smaller tidal amplitude (Paalvast *et al.*, 1998). Plant species composition in the area changed considerably, favouring more fast-growing species like *Urtica dioica* and *Epilobium hirsutum* (De Boois, 1982; Zonneveld, 1999).

Chemical changes

Besides the disappearance of the fresh-saline water gradient in the intertidal area, major changes in water quality occurred upstream as well. In the course of the Industrial Revolution the rivers started to function more and more as open sewers. Industrial effluent as well as domestic waste water were discharged unpurified into the rivers. Table 1 presents an overview of the historical concentrations (before 1900) of the major inorganic compounds of the river Rhine. River water pollution in the Netherlands reached its climax in the first half of the 1970s. The high concentrations of NH_4^+ and PO_4^{3-} measured at that time were a result of the untreated discharge of domestic waste water. The high oxygen demand of the river water led to bad water quality and mortality of fishes. Sulphate pollution in river water generally originated from atmospheric deposition of SO_x due to the combustion of fossil fuels, from acid mine drainage, from fertiliser use and from pyrite oxidation due to falling water tables. Fertiliser use, sewage treatment plants and anthropogenic NO_x deposition from traffic and agriculture caused the high nitrate concentrations that still remain present today. Remarkable is the salination (Na^+ and Cl^-) of the river water arising from discharge of the kali mines in the Alsace and to some extent also from domestic waste.

Slowly, more attention was paid to waste water treatment. Only in 1986 real international attention was drawn to the river water quality after the Sandoz event, when the Sandoz plant discharged a large quantity of polluted waste water into the river Rhine, ultimately resulting in the Rhine Action Programme (Van Dijk *et al.*, 1995; Nienhuis & Leuven, 2001). River water quality has improved substantially since the 1970s, but concentrations of Cl^-, NO_3^- and SO_4^{2-} remained high. Due to the pollution with nitrogen compounds of river water in Northwestern Europe, the riverine input of nitrogen to the North Sea

Table 1. Estimated concentrations of ions in the river Rhine at Lobith (mmol/L). Estimated from: Van der Weijden & Middelburg, 1989; Van den Brink *et al.*, 1991; Van den Brink, 1994; Ministry of Transport, Public Works and Water, 2008.

	historical	1970-1975	2000-2005
Na^+	0.2	4.9	2.3
K^+	0.12	0.19	0.12
Mg^{2+}	0.4	0.5	0.5
Ca^{2+}	1.2	1.4	1.8
NH_4^+	0.007	0.16	0.007
Cl^-	0.4	5.6	3.6
NO_3^-	0.02	0.2	0.2
SO_4^{2-}	0.36	0.86	0.60
HCO_3^-	2.6	2.4	3.0
PO_4^{3-}	0.002	0.010	0.002

is estimated on a 6 to 20 fold increase compared to the historical background values (Howarth *et al.*, 2006).

Not only river water quality changed in the past centuries, but also the floodplain soil quality. This was partly due to the sedimentation of polluted particles at the time of the worst river water quality, but also by the agricultural landuse of the floodplains, including the use of fertilizers (Howarth *et al.*, 1996; Grevilliot *et al.*, 1998; Lamers *et al.*, 2006).

Although river ecology has mainly focussed on the effects of geomorphological and hydrological changes in the riverine areas, it may be obvious that also changes in water and soil quality might have consequences for ecosystems in this area. Moreover, the influence of the river water stretches far beyond the riverine region. River water has been used as inlet water in the lower regions of the Netherlands to meet agricultural water level requirements in summer (e.g. Smolders & Roelofs, 1995). The inlet of this water has caused severe detoriation of peatlands (Smolders & Roelofs, 1995; Smolders *et al.*, 1996;Lamers *et al.*, 2002).

Biogeochemical processes upon flooding

This thesis especially focuses on processes that occur during flooding, and in places that are temporarily or (semi) permanently wet. These are also the places where interactions between soil and river water can be expected. Under dry conditions oxidation of organic matter in soil takes place aerobically. In that case micro-organisms use O_2 for decomposition. When inundated, transfer of O_2 to the soil is very slow, because diffusion through the waterfase is about four orders of magnitude slower than through the air (Laanbroek, 1990). Oxygen that is trapped as a gas in the soil pores, or is dissolved in the pore water, is depleted very rapidly. Subsequently, decomposition of organic matter takes place anaerobically, when other terminal electron acceptors are used as an alternative for O_2 (Table 2).

The consumption of H^+ in these redox processes implicates an increase in alkalinity. Theoretically, these redox processes take place at different redox potentials, ordered thermodynamically from high to low in the overview below. In the field, however, these processes partly overlap, meaning that often two and sometimes three of these processes might occur at the same moment. This is due to heterogeneity in soil, creating different micro-environments with different redox potentials. Moreover, these processes are performed by different micro-organism species, often using different electron acceptors and regularly competing with each other for certain forms of organic matter.

Table 2. Most important redox processes in water in the oxidation of organic matter, with examples of reactions (adapted from: Stumm & Morgan, 1981). CH_2O represents organic matter.

denitrification
$4\ NO_3^- + 5\ CH_2O + 4\ H^+ \rightarrow 2\ N_2 + 5\ CO_2 + 7\ H_2O$

manganese reduction
$2\ MnO_2 + CH_2O + 4\ H^+ \rightarrow 2\ Mn^{2+} + CO_2 + 3H_2O$

nitrate reduction
$NO_3^- + 2\ CH_2O + 2\ H^+ \rightarrow NH_4^+ + 2\ CO_2 + H_2O$

iron reduction
$4\ Fe(OH)_3 + CH_2O + 8\ H^+ \rightarrow 4\ Fe^{2+} + CO_2 + 11\ H_2O$

sulphate reduction
$SO_4^{2-} + 2\ CH_2O + 2\ H^+ \rightarrow H_2S + 2\ CO_2 + 2\ H_2O$

methanogenesis
$2\ CH_2O \rightarrow CH_4 + CO_2$

decreasing redox potential

The alternative electron acceptors NO_3^- and SO_4^{2-} are found in high concentrations in the river water. As stated before, concentrations of both are elevated compared to the historical concentrations. Mn and Fe both are important components of the mineral soil. Under aerobic circumstances they are mainly present as oxides and hydroxides. In reduced form their solubility increases, causing mobility of these elements. For this reason, large concentrations of these elements are often found at places with groundwater discharge. In anaerobic groundwater streams, they are carried along with the water, but in contact with oxygen Fe^{2+} and Mn^{2+} are oxidised again and accumulate. Due to the decrease of groundwater discharge towards the floodplains, it might be expected that the concentrations of Fe and Mn in the floodplain soils might become lower (Lamers *et al.*, 2006). Under anaerobic conditions organic matter can be decomposed in the last step to CH_4 and CO_2 in absence of alternative electron acceptors.

Eutrophication

Eutropication is defined as the increase of plant nutrient availability in an ecosystem. Eutrophication generally causes the disappearence of -often characteristic- plant species, because they are outcompeted by faster growing -generally more common- species. In aquatic systems eutrophication can cause algal blooms, also leading to the disappearence of characteristic higher plant species. High nutrient loads do however not always affect plant communities in the riverine area. In highly dynamic systems, such as frequently flooded sites within floodplains, disturbance events rather than competition of plants for nutrients determine the composition of the vegetation (Grime 1974; 1977). In that case, a higher connectivity of floodplains with the river water may lead to a higher biodiversity (Lamers *et al.*, 2006). Plant species that are able to survive under the restored disturbance are ruderal plant species; these are characterised by a life cycle adapted to high dynamic situations, such as sedimentation and long-term flooding. However, in less dynamic places within the floodplains, often located somewhat further from the river bed, eutrophication may enhance biomass production and loss of biodiversity if the increased nutrient is limiting biomass production. For wetlands and (semi)terrestrial plant communities the nutrients N, P and K are the elements most often mentioned as limiting nutrients (e.g. Koerselman & Meuleman, 1996; Van Oorschot *et al.*, 1998; Olde Venterink *et al.*, 2001; Beltman *et al.*, 2007).

Accordingly, the increase of nutrient concentrations in the river water and river sediment may cause loss of biodiversity in less dynamic parts of riverine landscapes. Today, especially the concentration of nitrate is still elevated in the river water, whereas in floodplain soils phosphate concentrations and phosphate saturation are expected to be elevated due to the use of fertilisers. It is, however, not only the elevated concentrations of nutrients causing eutrophication effects. Also the anaerobic decomposition processes themselves and the interaction with river water quality may cause the accelerated release of nutrients which were unavailable for plants before (‘internal eutrophication’). Microbial decomposition rates of organic matter are highly dependent on temperature, for which reason the temperature during the flood might form an important factor in the severity of the eutrophication. Anaerobic decomposition has a direct influence on nutrient cycling, especially on the P cycle. In floodplain soils a considerable part of phosphates are bound to iron(III) (hydr)oxides. When these are reduced as alternative electron acceptors, affinity for phosphate binding decreases, mobilising part of the phosphate (Patrick & Khalid, 1974; Ponnamperuma, 1984). The reduction of sulphate also influences the P cycle. In the reduction of sulphate, sulphide is formed, which has a very high affinity for iron. When this sulphide binds to iron, even more phosphate that was originally adsorbed onto iron particles, can be mobilised (Caraco *et al.*, 1989;

Smolders & Roelofs, 1993; Roden & Edmonds, 1997; Lamers *et al.*, 1998). This internal eutrophication as a result of increased sulphate concentrations, as well as the toxicity of sulphide, have been shown to form the major causes of deterioration of fen systems in the Netherlands (Smolders & Roelofs, 1995; Smolders *et al.*, 1996; Lamers *et al.*, 2002). It can however be questioned whether these processes play the same role in the riverine floodplains, where mineral soils and different plant communities dominate. The present study aims to unravel the role of these processes in floodplains.

Toxicity

Most toxicity studies in floodplains focus on organic pollutants and heavy metals. Many reduced inorganic substances, such as hydrogen sulphide, iron, manganese and ammonium are, however, potentially toxic for plants as well. For hydrogen sulphide, even very low concentrations can be phytotoxic (Smolders & Roelofs, 1996;Van der Welle *et al.*, 2006; Van der Welle *et al.*, 2007). By forming FeS_x (s), iron and sulphide can detoxify each other (Van der Welle *et al.*, 2006), but if iron concentrations are low and (hydrogen) sulphide concentrations are high, this can also lead to iron deficiency in plants. High concentrations of reduced, dissolved iron at concentrations present in the field can however become toxic (Snowden & Wheeler, 1993; Lucassen *et al.*, 2000). Dissolved manganese can be toxic too. Although not much is known about manganese toxicity, it is generally assumed that concentrations in the field are below effect levels. Ammonium, although being a nutrient, can be toxic if the nitrate:ammonium ratio is low at low pH's (Lucassen *et al.*, 2003). Under anaerobic circumstances, nitrification is blocked, leading to accumulation of ammonium, whereas the concentration of nitrate drops due to denitrification. Generally, the pH and alkalinity of floodplain pore water are high during flooding and the acid neutralising capacity is sufficient to prevent a drop in pH during subsequent dry conditions, so ammonium toxicity is not expected to play a large role in vegetation development in floodplains. In the studies presented in this thesis possible sulphide accumulation and its results are examined in flooding experiments. The potential toxicity of iron is briefly discussed in Chapter 7.

Another important potential phytotoxin, already present in the river water, is chloride. In the brackish water areas, plant communities consist of species adapted to a high salinity, making them characteristic for these circumstances. In the fresh water areas, characteristic plants might be harmed by the increase of chloride concentrations in the river water (Van den Brink *et al.*, 1991;Van den Brink & Van der Velde, 1993).

Present and future aims and policies in river nature management

For a long time attention of nature conservationists for riverine floodplains has been low. The rivers were mainly looked upon as places with a natural beauty and a high cultural value (e.g. Thijsse, 1938). This changed in 1987 when Plan Ooievaar ('Plan Stork') (De Bruin *et al.*, 1987) was presented. The authors of this plan won a designers contest for a new vision on spatial planning of the riverine area. They presented an integral plan in which they focussed on natural, especially geomorphological, processes as driving forces behind nature development. They chose the historical, pre-occupational, situation as a reference for newly to develop nature reserves. Because a geomorphologically highly dynamic situation in the straitjacketed riverine area was often not feasible (De Bruin *et al.*, 1987), and because of the increased insight in the important role of large herbivores in the past in the maintenance of open spaces in the landscape (Vera, 1997), it was chosen to use these types of animals to create a more dynamic and open landscape. Since the introduction of Plan Ooievaar most of the new nature reserves along the rivers follow this process orientated, rather than output orientated, vision in their management (Nienhuis *et al.*, 2002).

In the winters of 1993 and 1995 alarmingly high water levels occurred in the rivers Rhine and Meuse. In 1995, these water levels were so high that a quarter of a million people had to be evacuated (Nienhuis, 2006), because it was unclear whether the dikes would withstand the water pressure. Awareness rose that the present system of dikes might not be sufficient to prevent floods in future, especially because incidentally higher water levels were expected as a result of global climate change (Christensen & Christensen, 2003). Up till then, the dikes were occasionally raised to annul the effects of the sedimentation in the floodplains as well as the subsidence of the land behind the dikes due to drainage. Now, it was recognised that this would not be a sustainable solution anymore. The government launched the 'Space for the River Plan' ('Ruimte voor de Rivier') (Ministery of Transport, Public Works and Water, 2000). In this plan a range of measures were introduced to overcome floods in future. Measures consisted, apart from raising the river dikes, of the lowering of the floodplains, lowering of the river bed, removing of groins, creation of side channels, replacement of dikes in river bottle necks, the introduction of water storage areas and the retention of water in upstream areas. The increasing connectivity of the floodplains with the river water, and the departure of agriculture from the floodplains, creates opportunities for the creation of new nature reserves, but might however also increase the negative interactions, as hypothesized above, between river water and soil quality on the development

of target plant communities. It was one of the aims of the project, where this thesis participates in, to detect such constraints for a sustainable development of nature in these areas.

After the closure of the Haringvliet, public awareness about the ecological losses in the intertidal area rose (Paalvast *et al.*, 1998; Smits *et al.*, 2006). In 1994 it was therefore decided to allow some tidal influence in the Haringvliet again in future. Partial opening of the sluices is now planned for 2010. As the expected tidal amplitude and salinity resulting from this measure differ from the historical situation, and agricultural land in this area will also be transformed into nature reserves, the effects on biogeochemical processes and on vegetation development are still not clear.

Scope of the project, thesis outline and hypotheses

This study is financed by the TRIAS programme of NWO (The Netherlands Organisation for Scientific Research). The project 'Biogeochemical constraints for sustainable development in riverine regions' (project number 835.80.010) was carried out together with Martijn Antheunisse (Utrecht University) and Marzia Miletto (NIOO-KNAW). Within the project, the focus of Martijn Anteunisse's study was especially on landscape ecology and nitrogen dynamics (Antheunisse, 2006). Marzia Miletto examined the role of sulphate reducing prokaryotes in the riverine region (Miletto, 2007).

The research presented in this thesis examines the biogeochemical processes influencing eutrophication and toxicity, as outlined in the preceding sections, in riverine floodplains in the Netherlands. This research especially focusses on the effects of the changes in water quality, in particular the high sulphate concentrations, and in soil quality on vegetation development in the scope of the present and future river management policies and expected global climate change.

Global climate change is expected to increase both temperature and the risk of summer floods. This implicates that floods may occur at higher temperatures. In **Chapter 2** the influence of temperature on these processes is studied in a mesocosm flooding experiment. It is hypothesized that the higher temperature during floods would speed up anaerobic decomposition processes, which would lead to higher phosphate release, especially in the presence of high sulphate concentrations. These hypotheses were tested by flooding sods from a species rich riverine hay meadow at two different temperatures either with sulphate-rich or with sulphate-poor water. Subsequently the sods were allowed to dry out, enabeling the study of the effects of flooding and subsequent dessication.

Chapter 3 focusses on the interactions between the increased sulphate concentration in the river water and sediment quality. The concentrations of iron and organic matter were expected to form key-factors in the effects of flooding with sulphate-rich river water. In soils that are rich in iron, the iron was assumed to sufficiently bind sulphide formed in anaerobic respiration, whereas in soils poor in iron sulphate reduction may lead to sulphide toxicity. It was also hypothesized that the production of sulphide would lead to a higher mobilisation of phosphate due to the binding of sulphide to iron, than in soils flooded with sulphate-poor water. As organic matter is the electron donor in the reduction of sulphate, it might be expected that in soils lacking organic matter, the effects of sulphate pollution would be less severe. These hypotheses were tested in a mesocosm experiment featuring three soils from floodplains of the river Meuse, differing in iron and organic matter concentration. Soils were flooded with water containig high or low sulphate concentrations. To test the effects of sulphide toxicity, *Glyceria maxima* was used as a phytometer. Additionally, the effects of sulphate and the different soil characteristics on the decomposition rate and on the production of CO_2 and CH_4 were tested in a complementary batch experiment.

Chapter 4 deals with the the predictability of phosphorus mobilisation upon flooding. When iron is reduced, phosphate bound to iron(hydr)oxides is released, but which factor determines the extent of the release? Is it determined by the history of land-use or by soil characteristics? This question is very important in the light of spatial planning, as the answer can help to prevent eutrophication when new areas are selected for water storage and wetland creation. The release of phosphorus was examined in flooded soil cores from four different floodplains along the rivers Rhine, Meuse and Overijsselse Vecht in the Netherlands and from two floodplains along the less impacted river Narew in Poland.

In the near future the Haringvliet sluices might be reopened to rehabilitate oligohaline conditions and tidal amplitude. **Chapter 5** describes a large outdoor mesocosm experiment in which the effects of the renewed salinity and tide were tested in a full factorial design. It was hypothesized that the renewed tidal amplitude would change the redox potential of the soil resulting in a change in decomposition rate of the soil organic matter. The salinity of the water might generate cation exchange, whereas the high sulphate concentrations in the saline water might cause sulphide toxicity and phosphate release. Moreover, the high chloride concentrations might favour plant species adapted to high salinity above less characteristic species. We tested these assumptions on vegetated sods from a nature reserve and an agricultural land on the shores of lake Haringvliet.

An increase in nutrient availability will only affect plant communities if the nutrient in question is actually limiting biomass production -either of the vegetation as a whole or of single species. As there are indications that nutrient limitation plays a minor role in vegetation along eutrophicated rivers (Spink *et al.* 1998; Antheunisse *et al.* 2006), we were interested whether this assumption would also apply to the rare target plant community *Fritillario-Alopecuretum*, which is found in the floodplains of the river Overijsselse Vecht. In **Chapter 6** a greenhouse fertilisation experiment is described, in which the effects are studied of addition of N, P and K on the biomass production of the vegetation as a whole, as well as on different plant families, also enabling identification of changes in vegetation composition as a result of nutrient addition. Measurements of internal nutrient concentrations and their ratios made it possible to relate changes to nutrient addition and biomass production.

Finally, the results of the preceding chapters are dicussed in the synthesis, **Chapter 7**, and the conclusions about the implications of the results of the present study for nature management in the riverine floodplains are presented.

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2

Effects of winter versus summer flooding and subsequent desiccation on soil chemistry in a riverine hay meadow

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Geoderma 145 (2008): 84-90

Abstract

Flooding of riparian meadows along rivers leads to a switch to anaerobic soil respiration, causing iron reduction and a corresponding release of phosphate. In addition, pollution of river water with sulphate may lead to higher phosphate release as a result of sulphide-iron interactions. As global climate change is expected to increase both temperatures and the risk of summer flooding, floods may occur at higher temperatures, leading to faster anaerobic processes in soils. In a mesocosm experiment we tested the effects of flooding with or without 1 mmol L^{-1} SO_4^{2-} at two temperatures on sods from a riverine hay meadow. In the control treatment, the water level was kept 10 cm below the soil surface. After four weeks at 5°C, the temperature was changed to 20°C, mimicking the effects of summer flooding. After seven more weeks, all sods were allowed to dry out. In the inundated sods, redox potential dropped during flooding, leading to higher concentrations of Mn^{2+}, Fe^{2+}, PO_4^{3-}, NH_4^+ and Ca^{2+} and a higher alkalinity of the soil pore water. Upon desiccation, redox potential increased immediately, leading to the oxidation of Mn^{2+}, NH_4^+ and Fe^{2+} and causing immobilisation of PO_4^{3-} and a temporary drop in pH. Inundation at 20°C resulted in a much faster release of Mn^{2+}, Fe^{2+}, PO_4^{3-} and Ca^{2+} and a higher acid consumption compared to flooding at 5°C. Reduction of the added sulphate did not lead to additional mobilisation of phosphate through competition with the produced sulphide for binding to iron, because of the high iron concentration in the soil, which is characteristic of many floodplains. It is concluded that seasonality of flooding determines accumulation rates of potential phytotoxins and the release rate of phosphate, which has important implications for floodplain management.

Introduction

Upon flooding or waterlogging of a soil, oxygen depletion leads to a switch from aerobic to anaerobic respiration. As a result, potentially toxic substances such as Fe^{2+}, NH_4^+ and S^{2-} may accumulate. Additionally, the reduction of Fe^{3+} can cause a release of PO_4^{3-} that was originally adsorbed to Fe(hydr)oxide particles (Patrick & Khalid, 1974; Ponnamperuma, 1984), as the affinity of P to Fe(III) is higher than to Fe(II). It has been shown that in fen and lake systems, extra phosphate can be released by the binding of S^{2-} to Fe (Caraco *et al.*, 1989; Smolders & Roelofs, 1993; Roden & Edmonds, 1997; Lamers *et al.*, 1998a). Sulphide could also chemically reduce iron, also leading to the release of phosphate (Sperber, 1958). Notwithstanding the high iron(hydr)oxide concentrations in floodplain soils compared to fen systems, sulphate reduction can still play a role in the release of phosphate during floods (Zak *et al.*, 2006; Loeb *et al.*, 2007). This might be of great importance, as many rivers are polluted with sulphate (e.g. Van der Weijden & Middelburg, 1989).

During the last decade, summer floods have tended to occur more frequently in Central and Eastern Europe than before. Severe summer and late-spring floods have occurred in the major rivers Odra, Vistula, Labe (Kundzewicz *et al.*, 2005) and Danube in the past ten years. Christensen & Christensen (2003) showed that global climate change may cause periods of heavy precipitation in Europe in summer, which may lead to more frequent summer floods, even though summers may become drier on average. Summer floods are known to have a greater impact on floodplain vegetation than winter floods. Koutecký and Prach (2005) showed that only 20% of the plant species present in a floodplain of the river Morova were able to regenerate from belowground parts after a flood in July 1997, and that recovery of the vegetation took several years. Van Eck *et al.* (2004; 2006) also found that floodplain species are less tolerant to summer floods than to winter floods and that tolerance to summer floods explains the distribution of plant species along elevation gradients in floodplains of the river Rhine. Less attention has been paid to the influence of seasonality of flooding on biogeochemical processes in the soil, although it is generally known that bacterial processes accelerate with increasing temperatures. We therefore expected that the above biogeochemical processes would take place at a higher rate during summer floods than during winter floods.

We selected a species-rich floodplain meadow to test the effects of temperature and water quality at the time of flooding. In a mesocosm experiment with intact soil-vegetation units (sods), we simulated winter flooding, succeeded by summer flooding and subsequent desiccation as in a summer drought.

Materials and Methods

Area description

In February 2003, 15 vegetated soil cores 18 cm in diameter and 20 cm in height were taken from a floodplain along the River Overijsselse Vecht in the Netherlands (N 52°33.3', E 6°7.3'). This specific meadow is being managed as a nature reserve by the State Forestry Service and is mown several times a year. The vegetation on this species-rich hay meadow can be characterised as a form of the *Fritillario-Alopecuretum pratensis* (Horsthuis *et al.*, 1994*)*. This vegetation is characterised by the presence of the endangered bulb species *Fritillaria meleagris*. The soil, characterised as a fluvisol, consisted of a mixture of clay and sand on a deeper peat layer. The vegetated topsoil which we used in our experiment consisted mainly of silt (>50%), but also contained clay (approximately 15%) and sand (approximately 10%), which formed the C-horizon. On top of this horizon, a distinct A-horizon (12% organic matter) of approximately 5 cm was present. The high Fe concentration (Table 1) in the soil originated from historical discharge of groundwater towards this meadow. This discharge is no longer present, due to drainage of surrounding fens and peatlands, which historically had a higher hydrological elevation. The soil was poor in inorganic N and K and had a low concentration of Fe- and Al-bound P, compared to other Dutch floodplain soils. The concentration of total P was, however, comparable to that in other, mostly eutrophic, floodplains. Eighty-six percent of the cation adsorption complex was occupied by Ca and Mg, preventing the soil from severe acidification.

Experimental set-up

The vegetated soil cores (sods) (19 plant species per 4 m^2), placed in pots perforated at the bottom, were hung in containers with a volume of approximately

Table 1. Soil characteristics in μmol g^{-1} dry weight. Organic matter content and base saturation in %, CEC in cmol+ kg^{-1}.

Organic matter	12
Amorphous Fe (oxalate)	459
Total Fe (digestion)	693
Fe- and Al-bound P (Ca-EDTA)	3.3
Total P (digestion)	24
NO_3^- (MilliQ)	0.43
NH_4^+ (NaCl)	0.12
K^+ (NaCl)	0.06
CEC	24
Base saturation Ca	82
Base saturation Mg	3.8

10 L with outflows at 5 cm above and 10 cm below the soil surface (Figure 1; Lamers *et al.*, 1998b). Each soil core received water of controlled quality from its own 10 L reservoir, from which it was pumped at a rate of 10 L week^{-1} to the top of the soil cores, where it infiltrated. The surplus water was discharged either from the outflow at 5 cm above or that at 10 cm below the soil surface. Three rhizon samplers (Rhizon SMS - 5 cm, pore size 0.1 µm, Rhizosphere Research Products) were used to collect soil pore water.

The soil cores were kept in a climate room at 5°C with a day/night regime of 8 hours light (approximately 100 µmol m^{-2} s^{-1}) and 16 hours dark. After one week of acclimatisation, soil cores received one of the following treatments (n=5): (1) inundation up to 5 cm above the soil surface with sulphate-rich artificial river water, (2) inundation up to 5 cm above the soil surface with sulphate-poor river water, or (3) perfusion with the same sulphate-poor river water, but with the water table fixed at 10 cm below the soil surface. The composition of the artificial river water resembled the local river water quality. The characteristics of the artificial river water are listed in Table 2. After 4 weeks, temperature was increased to 20°C during the daytime and 15°C at night to imitate summer flooding. The day/night regime was changed to 12h/12h. After 7 more weeks, treatments were stopped and outer containers were emptied to let the soils dry out. For three weeks, the soil cores then only received artificial rain water

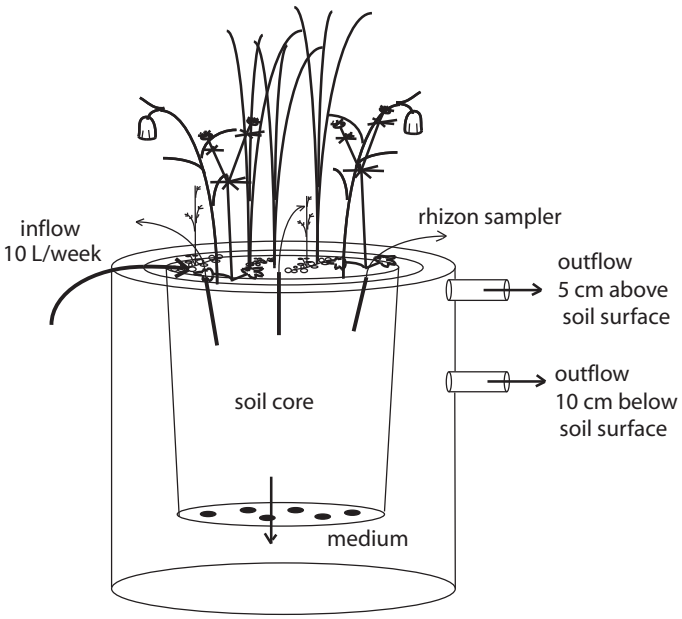

Figure 1. Experimental set-up.

Table 2. Composition of the artificial river water. Concentrations in µmol L^{-1}.

	SO_4-poor medium	**SO_4-rich medium**
Ca^{2+}	1300	1300
Mg^{2+}	250	250
K^{+}	250	250
Na^{+}	2000	4000
Cl^{-}	3300	3300
HCO_3^{-}	2000	2000
SO_4^{2-}	0	1000
HPO_4^{2-}	0.3	0.3
NO_3^{-}	35	35

(1650 mL in total over this period, containing 0.005 g/L seasalt (Wiegandt GmbH), 3.1 µmol L^{-1} KCl, 1.6 µmol L^{-1}, $CaCl_2.2H_2O$, 0.15 µmol L^{-1} KH_2PO_4, 67 µmol L^{-1} NH_4NO_3 and 87 µmol L^{-1} NH_4Cl).

Analysis of plant material

In order to determine the total concentrations of elements in plant material, 200 mg of homogenised and dried sample was digested for 17 minutes with 4 mL concentrated HNO_3 and 1 mL 30% H_2O_2 (Milestone microwave MLS 1200 Mega). Concentrations were analysed by ICP-OES (ICP, Spectroflame VML2).

Analysis of soil pore water

Each week, pore water samples were taken from the soil cores with the help of vacuumed glass bottles and analysed for pH, alkalinity, S^{2-}, NO_3^{-}, NH_4^{+}, PO_4^{3-}, Na^{+}, K^{+}, Cl^{-}, Ca^{2+}, Mg^{2+}, Fe^{2+}, Mn^{2+}, total dissolved S and total dissolved P. Samples from the three rhizon samplers of one soil core were pooled to reduce the effects of heterogeneity. At the end of the experiment, aboveground biomass was clipped and dry weight was determined after 24h drying at 70°C. Internal concentrations of P, Fe, Mn, Ca, Mg, Na, K and S were determined after digestion (see below). The pH was measured with a Radiometer Copenhagen type PHM 82 standard pH meter. Alkalinity was determined by titrating 10 mL of sample with 0.1 mol L^{-1} HCl down to pH 4.2. The concentration of free sulphide was determined in a 10 mL subsample, fixed with S^{2-} antioxidant buffer containing NaOH, NaEDTA and ascorbic acid. S^{2-} was detected using an S^{2-} ion-specific Ag electrode and a double junction calomel reference electrode (Thermo Orion) (Van Gemerden, 1984). For the ICP analyses (ICP-OES, Spectroflame VML2), concentrated nitric acid was added up to a concentration of 1.17% to the samples to avoid precipitation of elements. Samples were stored in polypropylene tubes at 4°C. For the other analyses, 0.12 g citric acid

L^{-1} was added to the samples, which were stored in iodated polyethylene bottles at -24 °C until further analysis. Total concentrations of Ca, Mg, P, Fe, Mn and S were analysed by ICP-OES (ICP, Spectroflame VML2). Total Fe and Mn measured in the pore water was regarded as Fe^{2+} and Mn^{2+}, since these are the dominant soluble species at the pH we measured. Total S was regarded as SO_4^{2-}, as, at the concentrations occurring in our samples, only a very small part will be in organic form and no free S^{2-} was found to be present. o-PO_4 and Cl^- (Technicon AutoAnalyser II), NH_4^+ and NO_3^- (+ NO_2^-) (Bran+Luebbe, TRAACS 800+ AutoAnalyser) were analysed colorimetrically using ammonium molybdate, ferriammonium sulphate, salicylate and hydrazine sulphate, respectively. We will consider this molybdate reactive phosphorus as phosphate. Na and K were measured by photospectrometer (FLM3 Flame Photometer, Radiometer Copenhagen) using lithium nitrate.

Soil analysis

Organic matter content of the sediments was determined by loss-on-ignition (4h, 550°C). In order to determine the total concentrations of elements in the soil material, 200 mg of homogenised and dried sample was digested for 17 minutes with 4 mL concentrated HNO_3 and 1 mL 30% H_2O_2 (Milestone microwave MLS 1200 Mega). Redox potential of the soil was measured weekly with a mV-meter with a platinum electrode and an Ag/AgCl reference electrode. Measured potentials were converted to redox potentials relative to standard hydrogen potential (E_H). Soil extractions were performed on fresh sediments and were corrected for moisture content afterwards, after drying (24h, 105°C). CEC and base saturation were determined by a triple sequential extraction of 5 g of fresh soil with a 0.1 M $BaCl_2$ solution, followed by extraction with a 0.020 M $MgSO_4$ solution. The CEC was calculated from the surplus of Mg in this extract. Relative base saturations of Ca and Mg were calculated from the concentrations of Ca and Mg, respectively, in the $BaCl_2$ extract, relative to the CEC. The concentration of amorphous Fe was determined by oxalate extraction (2.5 g of sediment shaken for 2h with 30 mL of a solution containing 16.2 g of $(COONH_4)_2.H_2O$ and 10.9 g of $(COOH_2).2H_2O$ per L (Schwertmann, 1964). To determine the Al- and Fe-bound P pools, a series of sequential P fractionations after Golterman (1996) was performed in duplicate: 2 g of field-moist soil was shaken twice (100 rpm) for 2 h with 25 mL 1M NH_4Cl to extract loosely bound and water-soluble phosphorus. The pellet was then shaken twice for 2 h with 30 mL 0.05 M Ca-EDTA to extract Fe- and Al-bound P. NH_4 and K concentrations were determined by NaCl extraction, using 50 mL 0.1 M NaCl per 17.5 g of soil. In the MilliQ (ultrapure water, 18.2 MΩ cm) extraction, 17.5 g of soil was shaken with 50 mL MilliQ to determine NO_3^- concentrations in soil. Digestions and extractions were measured as described above.

Statistical analysis

Net release rates were calculated by determining the regression coefficient per pot for a particular element in time during the cold or hot period. Rate differences in inundated sods were calculated by paired t-tests. Overall changes during the experiment, and the influence of the treatments, were assessed by repeated measures analyses (GLM) using log (x+1) transformed data. Significance was accepted at the 0.05 level. Effects of treatments on aboveground biomass and internal element concentrations were calculated by ANOVA using log (x+1) transformed data.

Results

Figure 2 shows the ion concentrations measured during the experiment. Table 3 lists the results of changes in the parameters over time and the effects of the treatments. Concentrations of Cl^-, which is useful as an inert tracer, show that the artificial river water had already fully penetrated the soil during the first week of flooding. During the flooding period at 20°C, Cl^- concentrations rose above those in the artificial river water, due to evaporation. At the end of the inundation period, Cl^- concentrations had increased to up to 1.5 times that in the artificial river water.

Table 3. P-values of the effects of time and time * treatment interactions (within-subject effects) and treatment effects (between-subject effects) (GLM for repeated measures). Different letters indicate differences between treatments for a particular element (Tukey post-hoc test). Due to insufficient values in week 12, results from this week were not included in the analysis, except for the redox potential measurements. For the same reason, the results of week 14 were omitted from the analysis for Fe, Mn, Ptotal, Ca and Mg.

	time	time* treatment	treatment	non-inundated	inundated -SO_4	inundated +SO_4
PO_4^{3-}	0.000	0.021	0.000	a	b	b
P_{total}	0.000	0.000	0.000	a	b	b
NO_3^-	0.000	n.s.	n.s.	a	a	a
NH_4^+	0.007	n.s.	0.007	a	ab	b
K^+	0.000	n.s.	n.s.	a	a	a
Fe^{2+}	0.000	0.000	0.000	a	b	b
Mn^{2+}	0.000	0.000	0.000	a	b	b
Ca^{2+}	0.000	0.000	0.000	a	b	c
Mg^{2+}	0.000	0.000	0.003	a	a	b
SO_4^{2-}	0.000	0.000	0.000	a	b	c
Cl^-	0.000	n.s.	n.s	a	a	a
pH	0.000	0.000	0.024	a	a	b
alkalinity	0.000	0.000	0.000	a	b	c
redox potential	0.000	0.000	0.000	b	a	a

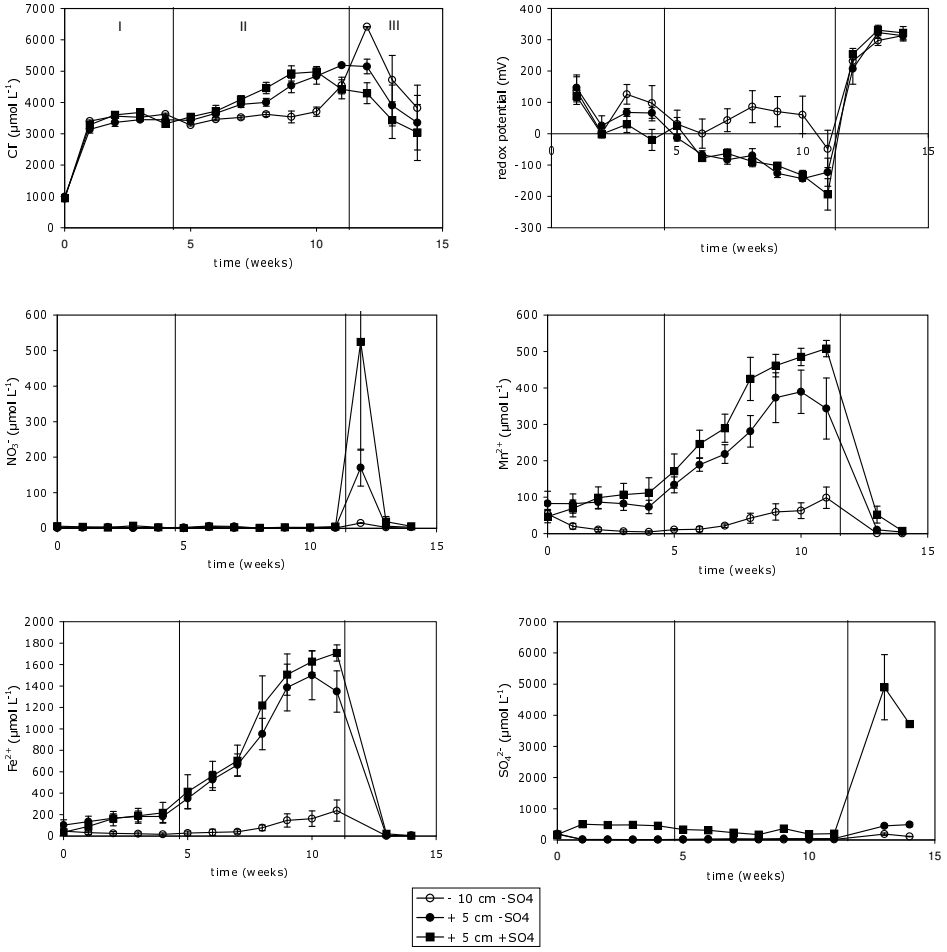

Figure 2. Soil pore water characteristics during the cold flooding period (I), the subsequent warmer flooding period (II) and the warm period of drought (III). Squares symbolise sods receiving sulphate-rich water; circles symbolise sods receiving sulphate-poor water. Open symbols represent control sods with the water table at 10 cm below the soil surface during the flooding period; closed symbols represent inundated sods with the water table at 5 cm above the soil surface during the flooding period. Error bars represent standard error of the mean.

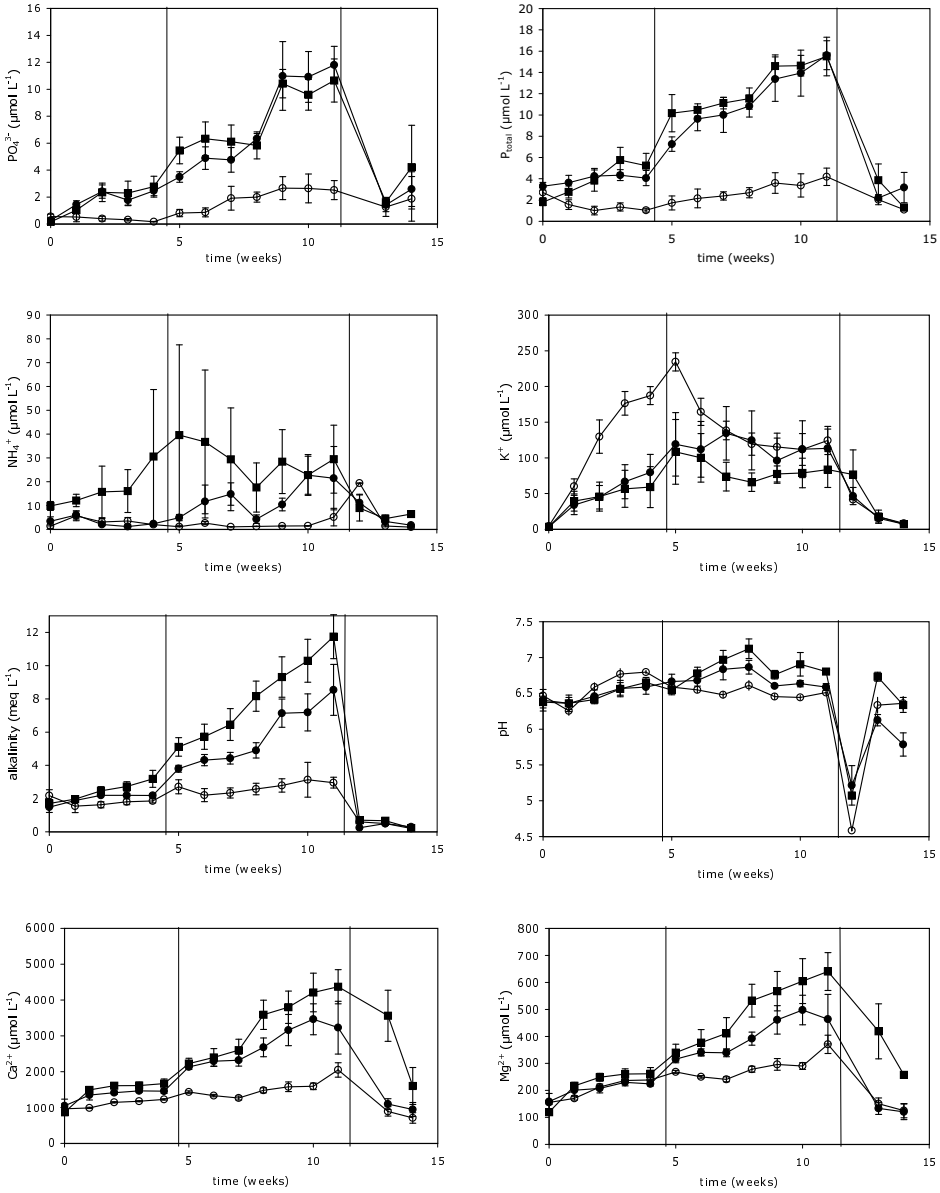

Figure 2. (continued)

After inundation, the redox potential dropped steadily in the inundated sods, from +140 mV at the start of the inundation to approximately -150 mV in the last week of flooding. During this decrease, the concentrations of Fe^{2+}, Mn^{2+}, PO_4^{3-}, Ca^{2+} and NH_4^+ and the alkalinity increased. NO_3^- concentrations remained low, below the detection limit (3 µmol L^{-1}) in most of the measurements. Fe^{2+}, Mn^{2+} and PO_4^{3-} in the soil pore water showed particularly large increases. Fe^{2+} concentrations rose from about 70 µmol L^{-1} at the start of the experiment to 1500 µmol L^{-1} at the end of the inundation period (a 21-fold increase), while Mn^{2+} rose from 65 to 430 (7-fold) and phosphate from 0.2 to 11 µmol L^{-1} (55-fold). Concentrations of Fe^{2+}, PO_4^{3-} and NH_4^+ also increased in the sods with the water level at 10 cm below soil surface compared to the increase in Cl^-, but the increase was much smaller than in the inundated sods.

During the warmer period, rates of biogeochemical process increased significantly. In the inundated sods, the net release of Fe^{2+} to the soil pore water was 7 times higher (32 µmol per liter pore water per day; p= 0.000) than during the colder period. Net PO_4^{3-} release rates were 3 times higher (0.18 µmol L^{-1} d^{-1}; p= 0.007), Ca^{2+} rates 2 times (45 µmol L^{-1} d^{-1}; p=0.002) and Mn^{2+} rates 6 times (6.9 µmol L^{-1} d^{-1}; p=0.001). Alkalinity production (acid consumption) was 9 times higher on average (1.1 meq L^{-1} d^{-1}; p=0.000). Concentrations of SO_4^{2-} in the pore water of the sods inundated with the SO_4-rich river water were 1.9 times higher during the colder inundation period than during the warmer period (p=0.005), although they were both significantly lower than the concentration of 1000 µmol L^{-1} we had added. When the cold period is compared with an equal time span of the warmer period (i.e. the first four weeks), the net release rates of phosphate did not differ significantly anymore, due to the temporal drop that we measured in the release rate during the fourth week of the warmer period. Release rates of total P did however still show the same trend as observed for phosphate when considering the complete warmer period (0.2 µmol L^{-1} d^{-1}; p=0.056; 3 times higher than in the colder period).

The sods receiving sulphate-rich river water showed higher concentrations of Na^+ and SO_4^{2-}, Ca^{2+} and Mg^{2+} in the soil pore water than the sods receiving sulphate-poor water. Concentrations of S^{2-} remained below the detection limit (1 µmol L^{-1}), just as in the other treatments. Alkalinity was higher in the treatment with sulphate than in the treatments without sulphate during the inundation period, and pH was slightly higher.

During desiccation, the redox potential increased from -150 to +300 mV. This led to a severe drop in pH, from an average of 6.6 to 5.0 in the first week after desiccation. During the following weeks, however, pH recovered to an average of 6.3. After desiccation, Fe^{2+}, Mn^{2+} and PO_4^{3-} concentrations and alkalinity in the soil pore water decreased and remained low. In the first week of desicca-

tion, NO_3^- concentrations showed a steep increase in the formerly inundated soils, up to values of 1000 µmol L^{-1}. In the subsequent weeks, concentrations of NO_3^- decreased again. SO_4^{2-} also showed an increase after desiccation, up to an average of 5000 µmol L^{-1} for the soils inundated with sulphate.

No effects of the treatments were found in the dry weight of the aboveground biomass at the end of the experiment. Some elements, however, did show significantly different concentrations in the aboveground biomass in the three treatments. Concentrations of Mn were 1.7 times higher in biomass from the inundated treatments treatments than in the non-inundated control ($p = 0.005$). The addition of SO_4^{2-} caused 1.7 times higher concentrations of S in the biomass of the sods treated with SO_4^{2-} ($p= 0.002$), and concentrations of the Na added with the SO_4 were 1.5 times higher in the biomass of the sulphate-treated sods than in the biomass of the sods inundated with the sulphate-poor water ($p = 0.039$). Fe concentrations in vegetation from the inundated sods were 1.6 times higher on average than in the controls, but the difference was not significant ($p = 0.375$). We found no effects of SO_4^{2-} addition on the Fe concentration in plant material.

Discussion

Redox processes

Despite the fact that the inundated sods were flooded with the same water quality as the control sods with the water table fixed at 10 cm below surface, they contained much higher concentrations (2-40 times) of PO_4^{3-}, NH_4^+, Fe^{2+}, Mn^{2+}, Ca^{2+} in their pore water, and had a higher alkalinity. The higher availability of Fe and Mn was also reflected in increased concentrations of these metals in the plant tissue. The PO_4^{3-} concentrations reached after flooding were in the same order of magnitude as recorded in other studies (Loeb *et al.,* 2007; Zak & Gelbrecht, 2007), although values 10 times higher have also been described for more heavily loaded floodplain soils (Kleeberg & Schlungbaum, 1993). The higher water level in the inundated sods clearly decreased the redox potential compared to the non-inundated sods. In these treatments, organic matter was decomposed anaerobically by the reduction of Mn^{4+} (resulting in increased concentrations of soluble Mn^{2+}) and of Fe^{3+} (resulting in increased concentrations of soluble Fe^{2+}), and by the reduction of SO_4^{2-} to S^{2-}. The use of NO_3^- as an electron acceptor may have contributed less to the decomposition of organic matter, due to the low concentrations of NO_3^-. Concentrations of Fe^{2+} in the pore water rose to 1700 µmol L^{-1}, which is in the same range as recorded in other wetland soils after 10 weeks of flooding (Zak & Gelbrecht, 2007). Note

that the reduction of Mn^{4+}, Fe^{3+} and SO_4^{2-} occurred simultaneously, indicating heterogeneity of the soil.

Van der Welle *et al.* (2007) found in a laboratory experiment that *Caltha palustris*, a species present in the field site from which we took our sods, already had reduced growth on peat soils at an Fe^{2+} concentration of 350 µmol L^{-1}. In a field survey, Lucassen *et al.* (2000) found clear symptoms of iron toxicity in *Glyceria fluitans* at pore water Fe^{2+} concentrations of 2600 µmol L^{-1}. It can therefore be said to be quite possible that iron toxicity occurs in the field that we examined and that toxic levels are reached much sooner at higher temperatures than at colder temperatures.

Our results show that water level, by determining redox potential, also formed a key factor for the availability of NH_4^+ and PO_4^{3-}. The anoxic circumstances in the soils led to inhibition of nitrification, and decomposition of organic material therefore led to accumulation of the NH_4^+ produced. As regards the extremely low nitrate concentrations we measured, it is unlikely that the accumulated NH_4^+ originated from dissimilatory reduction of nitrate to ammonium. After desiccation, NH_4^+ was immediately oxidised to NO_3^-, resulting in high NO_3^- concentrations in the first week after desiccation. The high release of PO_4^{3-} during inundation can be attributed to the reduction of Fe^{3+} including iron from iron oxides and iron hydroxides, which effectively adsorb PO_4^{3-} (Patrick & Khalid, 1974; Reddy *et al.*, 1999). Since the affinity of PO_4^{3-} for Fe(II) compounds is much smaller, reduction of Fe^{3+} leads to a release of PO_4^{3-} increasing the availability of the nutrient in pore water and, by diffusion, in the surface water (Ponnamperuma, 1984). The actual mobilisation of P to the pore water is determined by the concentration of amorphous Fe and its saturation by P (Young & Ross, 2001). The release of P from the soil pore water to the surface water is decisive for the eutrophication of surface waters and the discharge of P by river water. Since the sediment-water interface often also forms the interface between the anaerobic and aerobic environments, and the top layer of the sediment may also be oxic, the release of P to the water layer cannot be directly predicted by the release of P to the pore water. Reoxidised Fe adsorbs phosphates diffusing towards the surface water and in this way prevents release to the water layer (e.g. Boström *et al.*, 1988; Moore & Reddy, 1994). Release of P to the surface water has been reported to be determined by a number of factors, including the Fe:P ratio in the pore water (Smolders *et al.*, 2001), the Fe:P ratio in the aerobic top layer of the sediment (Jensen *et al.*, 1992), wind and bioturbation, precipitation with $CaCO_3$ (Boström *et al.*, 1988) and by the phosphate concentration in the soil pore water itself (Young & Ross, 2001).

The drought period showed large differences with the preceding period, as all substances whose reduction was described above were oxidised again. Even

the non-inundated treatment manifested a different pore water composition during the dry period than before. Although the differences for Mn^{2+} and Fe^{2+} concentrations and alkalinity between this dry period and the period preceding were not as large as in the inundated sods, they still showed that when the water level is set at -10 cm, the anaerobic processes deeper in the soil still play an important role for the mobility of nutrients and metals.

High increases of concentrations of Fe^{2+} and Mn^{2+} indicated increased anaerobic respiration by Fe^{3+} and Mn^{4+} reduction at higher temperatures. In addition, the lower concentrations of soluble total S - this was mainly SO_4^{2-}, because no S^{2-} was present - in the pore water during the warmer period, suggested a higher sulphate reduction rate. The higher temperature also led to higher increases of concentrations of PO_4^{3-}. Increased PO_4^{3-} availability at the temperatures we applied was of the same order of magnitude as found by Zak *et al.* (2006) for a floodplain soil and by Koerselman *et al.* (1993) for peat soils. It is also in line with Van 't Hoff's rule, according to which the turnover rates in our experiment should increase by a factor of 3. Jensen and Anderson (1992) also found that in shallow, eutrophic lakes, phosphate release from the sediment to the water phase is much higher at higher temperatures, due to the reduced P binding capacity of the thinner oxidised layer at the sediment-water interface.

Acid-base processes, desorption and cation exchange

Since the reduction of Mn^{4+}, Fe^{3+} and SO_4^{2-} are acid-consuming processes, alkalinity increased during inundation. The higher alkalinity in the pore water of the inundated sods treated with sulphate can be attributed to the reduction of SO_4^{2-}, which generates a significantly higher alkalinity than that in the inundated sods without sulphate. Upon desiccation, the oxidation of NH_4^+, Mn^{2+}, Fe^{2+} and S^{2-}, as indicated by the results, resulted in a high acid production, leading to a drop in pH during the first week. The re-establishment of the circumneutral pH in the subsequent weeks can be explained by the buffering effect of bicarbonate and subsequently by cation exchange of protons from the solution with other cations from the cation adsorption complex (not shown by the results). After desiccation, NO_3^- concentrations rose to values of 5000 µmol L^{-1}, whereas the concentrations of NH_4^+ in the soil pore water just before desiccation did not exceed 100 µmol L^{-1} in any of the sods. Therefore, it seems likely that the nitrified NH_4^+ originates mostly from the adsorption complex (Lucassen *et al.*, 2006), either by desorption induced by the low ammonium concentrations in solution, or by cation exchange.

The Ca^{2+} concentrations in the soil pore water of the inundated sods greatly exceeded the concentrations of Ca^{2+} supplied with the artificial river water. The

sods with added SO_4^{2-} showed an even higher Ca^{2+} and Mg^{2+} release than those without added SO_4^{2-}. There are two possible explanations for the release of Ca^{2+} and Mg^{2+}. In the inundated soils CO_2, originating from decomposition of organic matter, could be trapped in the soil and could cause dissolution of $CaCO_3$ and $MgCO_3$, which could be present in the soil in small amounts, notwithstanding the low initial pH of the soil. In the sods receiving sulphate, decomposition of organic matter was increased by the higher availability of electron acceptors. Additionally, part of the raised concentrations of Ca^{2+} and Mg^{2+} compared to the non-inundated soils may be explained by cation exchange of the newly formed soluble cations generated in the soil itself during the inundation period, such as Fe^{2+}, Mn^{2+}, and NH_4^+. As shown in Table 1, initially 82% of the adsorption sites of the soil were occupied by Ca, Only a small part of the additional release of Ca^{2+} and Mg^{2+} in the inundated soils receiving sulphate could be explained by the exchange of Na^+, added as Na_2SO_4, against Ca^{2+} or Mg^{2+}, because a larger release by the monovalent Na^+ against the divalent Ca^{2+} and $Mg^{2=}$ would only be possible at higher concentrations of Na^+.

Effects of sulphate pollution

No free sulphide was detected in the soil pore water of the sods, as S^{2-} binds to Fe and the examined soil contained high concentrations of Fe. However, it was shown by the additional increase in alkalinity during flooding and by the release of sulphate after desiccation in the treatment with sulphate-rich water that sulphate reduction did take place. During dessication FeS_x was oxidised, leading to high sulphate concentrations in the pore water. Several other authors (Caraco *et al.*, 1989; Smolders & Roelofs, 1993; Roden & Edmonds, 1997; Lamers *et al.*, 1998a) have shown that the competition of S^{2-} – generated by the reduction of sulphate – with PO_4 for binding to Fe largely controls the release of PO_4 in fens and peatlands. Zak *et al.* (2006) and Loeb *et al.* (2007) showed that, depending on the soil type, inundation with sulphate-rich water may also increase the phosphate mobilisation in floodplain soils, which generally have higher soil Fe concentrations and lower organic matter content than fens and peatlands. The addition of sulphate to the river water did not have any effect on phosphate release in this particular soil. In this soil, the ratio of amorphous iron, which can be reduced by bacteria, to the amount of phosphate bound to it, was very high (Table 1). Hence, this soil is expected to be less sensitive to sulphate pollution (Zak *et al.*, 2006).

Conclusions

We showed that seasonality of flooding largely determines the accumulation of potential phytotoxins and the release of phosphate. This is not only important

for floodplain soils, but also at other locations where water is stored or retained as a measure in flood prevention strategies. Whether the release of phosphate to the soil pore water will actually lead to changes in the vegetation composition depends on the type of nutrient limitation. Although the vegetation type studied in the present experiment appeared to be limited by N in a greenhouse fertilisation experiment (Loeb *et al.*, accepted), greatly increased phosphate mobilisation to the surface water may lead to algal blooms and to eutrophication in downstream areas. The expected increase in the risk of summer floods will therefore have significant effects on floodplain biogeochemistry in terms of the potential for eutrophication and accumulation of potential phytotoxins, especially on fertilised meadows.

Acknowledgements

We would like to thank Lisa Kuijpers, Kim Kobes, Jelle Eygensteyn and Denise Buyk for their help with chemical analyses. We are grateful to the State Forestry Service (SBB) for their kind permission to collect sods from their property. We thank Jan Klerkx for linguistic improvements. This study was financially supported by the Netherlands Organisation for Scientific Research (NWO) as a part of the TRIAS programme (project 835.80.010).

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3

How soil characteristics and water quality influence the biogeochemical response to flooding in riverine wetlands

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Biogeochemistry 85 (2007): 289-302

Abstract

Although phosphate concentrations have been reduced, the rivers Meuse and Rhine are still polluted with sulphate, which most probably affects vegetation development in newly created riverine wetlands. The influence of flooding with river water rich in sulphate was tested on three soil types from floodplains of the river Meuse using flow-through and batch experiments. Soils were selected for contrasting concentrations of iron and organic matter and originated from a floating fen (iron-poor, organic), an alder carr (iron-rich, organic) and a clay pit (iron-rich, low in organic matter). Flooding induced mobilisation of phosphate. Sulphate only enhanced this effect in the alder carr soil, where sulphide and phosphate competed for binding to iron. Only in the floating fen soil did the addition of sulphate result in the formation of free sulphide, which reduced the growth of *Glyceria maxima*, serving as a phytometer. In addition, the floating soil started to sink, due to falling methane concentrations. In the different soil types methane production was hampered by the presence of more favourable electron acceptors such as sulphate in the water and iron(III) in the soil. It was concluded that the effects of inundation with sulphate-polluted water strongly depend on the soil type: under iron-poor circumstances, free sulphide may accumulate, leading to phytotoxicity, while in soils rich in iron, sulphide toxicity is prevented, but phosphate availability may be increased. In addition, shortage of easily degradable organic matter can limit the formation of potential toxicants such as ammonium, iron and sulphide. Results are discussed in terms of their implications for nature management.

Introduction

Although great efforts have been made since the 1970s to improve water quality in the rivers Meuse and Rhine, concentrations of many major ions are still elevated compared to their natural background values. Measures such as the introduction of wastewater treatment plants have effectively reduced the concentrations of ammonium and phosphorus in the river water, and decreased discharge from the industry and decreased atmospheric sulphur deposition have contributed to a decrease in concentrations of sodium, chloride and sulphate, respectively. However, today's average sulphate concentrations in the river Meuse still lie around 600 µmol L^{-1} (Ministry of Transport, Public Works and Water, 2006), whereas measured historical concentrations (before 1900) and calculated background concentrations for the Meuse and Rhine in the Netherlands are approximately 300 µmol L^{-1}. Concentrations of nitrate (around 50 µmol L^{-1}) are also still elevated compared to their background values (5 µmol L^{-1}) (Van der Weijden & Middelburg, 1989; Van den Brink *et al.,* 1991; Van den Brink, 1994). This article focuses on the effects of the elevated sulphate concentrations.

It is known that sulphate pollution can play an important role in the deterioration of freshwater wetlands, especially in peatlands (Roelofs, 1991; Smolders *et al.,* 1996, 2003; Lamers *et al.,* 1998, 1999, 2001, 2002a, b; Lucassen *et al.,* 2004). Under anaerobic circumstances, for instance during flooding, sulphate is reduced to sulphide in the absence of more favourable electron acceptors such as oxygen or nitrate. Free sulphide can be very toxic to wetland plant species, as has for instance been shown for *Stratiotes aloides* (Smolders & Roelofs, 1996) and *Caltha palustris* (Van der Welle *et al.,* 2007). However, sulphide has a high affinity for binding to iron, which decreases the availability of free sulphide and hence its toxicity (Van der Welle *et al.,* 2006). As the affinity to iron is higher for sulphide than for phosphate, the latter can be released during this process, causing eutrophication (Caraco *et al.,* 1989; Roden & Edmonds, 1997; Lamers *et al.,* 1998). This mobilisation of phosphate is added to the release of phosphate that takes place upon reduction of iron after the flooding of semi-terrestrial wetlands, such as floodplains. After inundation and the depletion of oxygen from the pore water, iron can be used as an electron acceptor for the oxidation of organic matter. This causes a partial release of the phosphate that was bound to iron(hydr)oxides under aerobic circumstances (Patrick & Khalid, 1974). It is, however, unclear if sulphate has similar detrimental effects on floodplain biogeochemistry.

Besides the phytotoxic and eutrophying effects of sulphate after reduction, sulphate is also known to inhibit bacterial methane production (Cappenberg, 1974; Abram & Nedwell, 1978; Lovley & Klug, 1983; Westermann & Ahring,

1987; Prieme, 1994; Scholten & Stams, 1995). It has been demonstrated that as a result of this, floating Sphagnum bog rafts, which depend on methane for floatation, sink under the influence of increased sulphate concentrations (Lamers *et al.,* 1999; Smolders *et al.,* 2002). As floating fen mats present in riverine systems also depend on methane for buoyancy (Hogg & Wein, 1988a; Swarzenski *et al.,* 1991; Sasser *et al.,* 1996), a similar effect of elevated sulphate concentrations can be assumed to exist, although this has never been reported. Floating mats form an important phase in terrestrialisation and hence in vegetation succession of peatlands (Tallis, 1973; Beltman, *et al.* 1996). They can be found in river deltas across the world, such as in the rivers Mississipi, Danube, Nile and Amazone (Sasser *et al.,* 1996; Hogg & Wein, 1988b).

During the last decade, many new wetlands have been created in the floodplains of large rivers, including the rivers Rhine and Meuse, either for the purpose of ecological restoration, or for flood risk reduction, or for a combination of both objectives. Plans for riverine wetland creation often ignore river water quality and soil quality, although it may be hypothesised that the effects of sulphate pollution that have been found in peatlands may also play an important role in riverine floodplains. Since concentrations of iron and of easily degradable organic matter are expected to act as key factors differentiating the effects of sulphate pollution, we chose to examine three different floodplain soils from the river Meuse, differing in iron and organic matter contents.

Materials and methods

Site description

Three different floodplain areas along the river Meuse in the Netherlands were selected for their soil characteristics. Figure 1 depicts the locations of the sites. The first soil (F) was taken from a floating fen at the bank of an old oxbow lake (the 'Oude Maas') anchored to the river dike (N 47°58'43.04'' E 3°18'56.38''). This lake discharges its water into the Meuse and only comes into direct contact with the river water during floods. The soil consists mostly of roots and plant litter. The vegetation type it supports, which is dominated by the fern *Thelypteris palustris*, has become very rare in Dutch floodplains. A vegetation relevé from 1954 shows that at that time, the oxbow lake was partly vegetated by *Stratiotes aloides* (Van Donselaar, 1961). The second soil (C) was taken from the shores of a recently dug clay pit (N 47°58'41.42'' E 3°18'58.26'') in the Romeinenweerd nature reserve. In this area, several small lakes have been excavated for the purpose of nature restoration and flood risk reduction. The excavated clay was used for the construction of flood protection systems in neighbouring towns. The lake from which we collected the

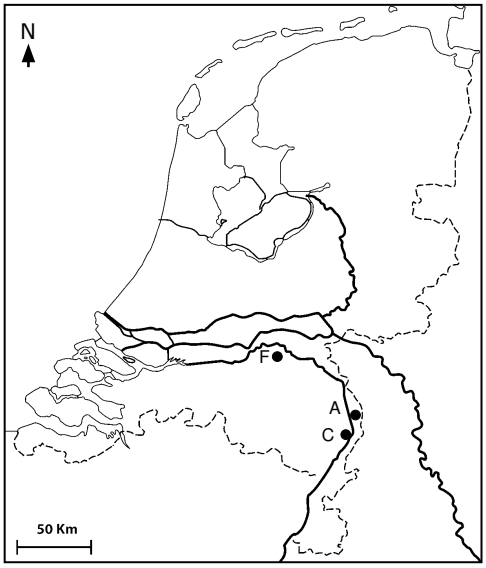

Figure 1. Location of the sampling sites within the Netherlands. A = alder carr, C = clay pit, F = floating fen.

soil, is a stagnant water body, which suffered severe eutrophication in the hot summer of 2003, when a bloom of cyanobacteria led to high mortality of fish and waterfowl (Pollux & Pollux, 2004). No vegetation was present at the spot from which we took the soil. The third soil (A) was taken from a black alder carr (N 47°58'42.27'' E 3°18'57.98''), called Heuloërbroek, within the winter bed of the river Meuse. The site receives discharge of iron-rich groundwater from higher situated areas. The top soil consists of litter, mainly from *Alnus glutinosa*.

Soil characteristics are summarised in Table 1. The clay soil was poor in organic matter (about 5%), while the other two soils were mostly organic. Both the alder carr and clay soils had a high concentration of iron, but the oxalate-extractable pool, which is regarded as the amorphous iron that is available for bacterial reduction, was much lower in the clay soil, both in absolute and in relative terms. The iron concentration of the floating fen soil was very low. Both total P and Olsen-extractable P were high in the alder carr soil.

Table 1. Soil characteristics.

Soil	Organic matter content (%)	Oxalate-extractable Fe (μmol g^{-1} dw)	Total Fe (μmol g^{-1} dw)	P-Olsen (μmol g^{-1} dw)	Total P (μmol g^{-1} dw)
Floating fen	83	50	100	3	30
Clay pit	5	104	610	1	24
Alder carr	52	950	1011	5	104

Experimental set-up

Aquarium experiment

Twenty-four 9 L aquaria were filled with a 10 cm layer (1/3 of the aquarium) of carefully mixed soil (4 replicates) (Figure 2) and kept in a water basin at a temperature of 20°C in a climate chamber at a light intensity of 200 µmol m^{-2} s^{-1} (Hortilux Schreder HPS 400 W) for 12 hours a day. Two 10 cm pore water samplers (Rhizon SMS -10 cm; Eijkelkamp Agrisearch Equipment, The Netherlands) were placed diagonally in the soil of each aquarium. A 5-10 cm layer of surface water collected in the field was added on top of the soil. After one week, the soils were further inundated with artificial river water containing either 1 mmol L^{-1} SO_4^{2-} (as Na_2SO_4) or no SO_4^{2-} (Table 2), from 10 L stocks, each connected to their own aquarium. The medium was pumped through the aquaria at a speed of 10 L per week. Three shoots of *Glyceria maxima* and 0.25 g of *Lemna minor*, collected in the field, were added to each aquarium as phytometers. Other plant species than *G. maxima*, *L. minor* or algae were carefully removed from the aquaria. Pore water samples were taken by connecting 100 mL vacuum bottles to the rhizon samplers. Samples of both pore water samplers were pooled per aquarium to diminish the effects of heterogeneity. Surface water samples were filtered (Whatman GF/C). After 12 weeks, the aboveground biomass of *G. maxima* and the floating *L. minor* and algae biomass were harvested. In the aquaria with the alder carr soil, algae were harvested more often, to prevent die-off of *G. maxima* from light limitation.

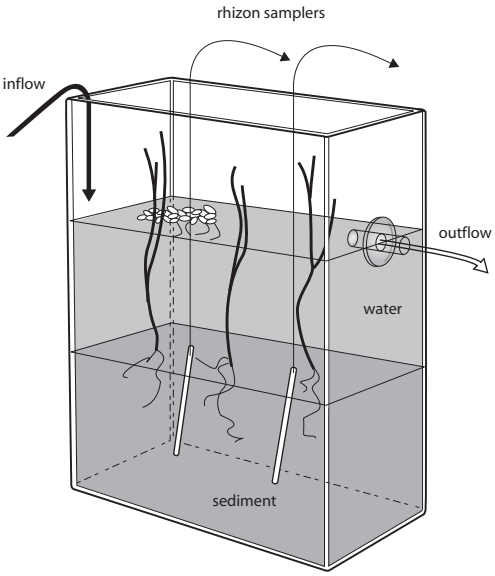

Figure 2. Experimental set-up. Picture: Lidwien van der Horst.

Table 2. Concentrations of macro-ions in the artificial river water treatments, with and without sulphate.

	Medium -SO_4 (µmol L^{-1})	Medium +SO_4 (µmol L^{-1})
Na^+	2000	4000
Ca^{2+}	1300	1300
Mg^{2+}	250	250
Cl^-	3100	3100
HCO_3^-	2000	2000
SO_4^{2-}	0	1000

Decomposition experiment

50 g portions of fresh soil were transferred to 350 mL glass bottles. The bottles were filled up to 170 mL with the same medium as used in the aquarium experiment and preflushed with N_2 to remove O_2 from the water. The headspace of the bottles was also flushed with N_2 before they were sealed with airtight rubber stoppers. We will refer to this soil and water suspension as slurry. Analyses were done in quadruplicate. Initial concentrations in the slurry water were determined by filling smaller bottles with 25 g of fresh soil and filling them up to 85 mL with medium. These bottles were shaken overnight. Slurry water samples were taken by pore water samplers (Rhizon SMS-5cm; Eijkelkamp Agrisearch Equipment, The Netherlands). Decomposition bottles were shaken in the dark at 20 ˚C. Samples from the headspace were taken twice a week with 1 mL syringes and were analysed instantly for CH_4 and CO_2. After 42 days, slurry water samples were taken and the experiment was ended. Potential production rates were calculated by linear regression of the net production in the linear phase of the production rate of CH_4 or CO_2. Potential decomposition rates were calculated in the same way, expressed as the C production rates in the linear phase of the concentration build-up of the sum of CH_4 and CO_2. This does not equal the sum of the potential CH_4 and CO_2 production rates, because of the difference in lag phase. Potential net release rates were calculated from the difference between the pore water concentrations at the start and the end of the experiment, divided by the time elapsed.

Chemical analyses

Immediately after collection of the pore water, the concentration of free sulphide was determined in a 10 mL subsample, fixed with S^{2-} antioxidant buffer containing NaOH, NaEDTA and ascorbic acid (Van Gemerden, 1984). S^{2-} was detected using an S^{2-} ion-specific Ag electrode and a double junction calomel reference electrode (Thermo Orion) (Van Gemerden, 1984). pH was measured with a radiometer Copenhagen type PHM 82 standard pH meter. Alkalinity was determined by titrating 10 mL of sample with 0.1 mmol L^{-1} HCl down to pH 4.2. For the ICP analyses, concentrated nitric acid was added up to a concentration of 1.17% to the samples to keep the oxidised iron in solution. Samples were stored in polypropylene tubes at 4°C. For the other analyses, 0.12 g citric acid/L was added to the samples. They were stored in iodated polyethylene bottles at –24°C until further analysis. Total concentrations of Ca, Mg, P, Fe and S were analysed by ICP-MS (X series, Thermo Electron Corporation) and ICP-OES (IRIS Intrepid II XDL, Thermo Electron Corporation). Total Fe measured in the pore water was assumed to be Fe(II), since this is the dominant soluble species at the pH values we measured. Total S was assumed to be SO_4 (if no sulphide was present), as, at the concentrations occurring in our soils, only a very small part will be in organic form. This was confirmed by parallel SO_4^{2-} analysis of part of the samples with methylthymol blue and $BaCl_2$ (Bran+Luebbe, AutoAnalyser 3, Digital Colorimeter). o-PO_4 (Technicon AutoAnalyser II), NH_4^+, NO_3^- (+ NO_2^-) and Cl^- (Bran+Luebbe AutoAnalyser 3) were analysed colorimetrically using ammonium molybdate, salicylate, hydrazine sulphate and ferriammonium sulphate, respectively. CO_2 and CH_4 were measured by infrared analysis (ABB Advance Optima Infrared Gas Analyser).

In order to determine the total concentrations of elements in plant and soil materials, 200 mg of homogenised and dried sample was digested for 17 minutes with 4 mL concentrated HNO_3 and 1 mL 30% H_2O_2 (Milestone microwave MLS 1200 Mega). C and N concentrations in the plant material (20 mg) were determined with a CNS analyser (Carbo Erla Instruments NA 1500).

Organic matter content of the soils was determined by loss-on-ignition (4h, 550°C). Soil extractions were performed on fresh soils and corrected for moisture content afterwards, after drying (24h, 105°C). The concentration of amorphous Fe, which is supposed to represent the fraction available for microbial Fe reduction, was determined by oxalate extraction (2.5 g of soil shaken for 2h with 30 mL of a solution containing 16.2 g of $(COONH_4)_2.H_2O$ and 10.9 g of $(COOH_2).2H_2O$ per L) (Schwertmann 1964) and plant-available P by bicarbonate extraction (5 g of soil shaken with 100 mL of 0.5 M $NaHCO_3$) (Olsen *et al.,* 1954).

Statistics

All concentrations were log (+1) transformed to improve their fit to a normal distribution, except for floatation measurements. All statistics were calculated with SPSS 13.0 (SPSS Inc., 2004). Effects of time series were tested with GLM repeated measures, using Greenhouse-Geisser corrections if terms of sphericity were not met in Mauchly's sphericity test. Effects of sulphate addition on the specific soil types were assessed with an independent samples T-test using data of the last measurement, unless stated otherwise. Effects of sulphate on concentrations and production rates of methane and on iron release were tested with 1-tailed tests, because the direction of the effect was already known. Differences between soil types were assessed by univariate ANOVA. Decomposition rates were calculated over the linear range of CO_2 or CH_4 production by linear regression. Significance was accepted at a confidence level of $P \leq 0.05$.

Table 3. P values of the time effects (time, time*soil interactions (time*soil), time*treatment interactions (time*treat) and time*soil*treatment interactions (time*soil*treat) of the pore water and surface water concentrations in the aquarium experiment, as tested by repeated measures (GLM). Bold values indicate $P \leq 0.05$.

	time	time*soil	time*treat	time*soil*treat
pore water				
P	**0.00**	**0.00**	0.55	0.83
Fe^{2+}	**0.00**	**0.00**	**0.01**	**0.00**
Ca^{2+}	**0.01**	**0.00**	0.59	0.36
NH_4^+	**0.00**	**0.00**	0.35	0.08
Cl^-	**0.00**	**0.00**	0.69	0.31
Na^+	**0.00**	**0.00**	**0.00**	0.12
K^+	**0.00**	**0.00**	0.83	0.96
SO_4^{2-}	**0.00**	**0.00**	**0.00**	**0.00**
PO_4^{3-}	**0.00**	**0.00**	0.25	0.48
Mn^{2+}	**0.00**	**0.00**	0.07	0.27
Mg^{2+}	**0.01**	**0.00**	0.57	0.56
pH	**0.00**	**0.00**	**0.04**	0.27
Alkalinity	**0.00**	**0.00**	**0.03**	0.42
surface water				
P	**0.00**	**0.00**	0.17	0.06
Fe^{2+}	**0.00**	**0.00**	0.06	0.39
Ca^{2+}	**0.00**	**0.00**	0.37	0.32
NH_4^+	**0.00**	**0.00**	**0.00**	**0.02**
Cl^-	**0.00**	**0.03**	0.40	0.65
Na^+	**0.00**	**0.00**	**0.00**	0.32
K^+	**0.00**	**0.00**	0.32	0.08
SO_4^{2-}	**0.00**	**0.00**	**0.00**	0.24
PO_4^{3-}	**0.00**	**0.00**	**0.03**	**0.01**
Mn^{2+}	**0.00**	**0.02**	**0.00**	0.06
Mg^{2+}	**0.00**	**0.00**	0.67	0.25
pH	**0.00**	**0.00**	0.06	0.12
Alkalinity	**0.00**	**0.00**	**0.01**	0.47

Table 4. Initial concentrations of major ions and elements in the pore water. Concentrations are given in μmol L^{-1}, alkalinity in meq L^{-1}. F = floating fen, C = clay soil, A = alder carr soil. Standard error of the mean is given in brackets. Significant differences between soils are indicated by different letters.

	F		C		A	
Na^+	762^a	(121)	1421^b	(17)	1502^b	(17)
Ca^{2+}	1027^a	(169)	5516^c	(199)	1780^b	(96)
Mg^{2+}	167^a	(26)	1060^c	(42)	343^b	(18)
Fe^{2+}	26^a	(7)	264^b	(52)	1570^c	(131)
Mn^{2+}	13^a	(4)	241^c	(18)	46^b	(3)
pH	6.3^a	(0.1)	7.1^b	(0.1)	6.5^a	(0.03)
NH_4^+	117^b	(19)	42^a	(12)	193^c	(68)
K^+	189^a	(28)	208^a	(15)	383^b	(6)
P	66^c	(10)	2^a	(0.2)	16^b	(2)
PO_4^{3-}	59^c	(10)	0.5^a	(0.1)	11^b	(2)
Cl^-	855^a	(51)	1369^b	(25)	2382^c	(32)
SO_4^{2-}	17^a	(6)	1903^c	(340)	440^b	(284)
alkalinity	1.8^a	(0.2)	7.7^c	(0.5)	3.8^b	(0.2)

Results

Aquarium experiment

Pore water and surface water

Table 3 summarises the results of the repeated measures analyses of the concentrations of elements and compounds in the pore water and surface water. From this table it can be seen that concentrations changed over time for all elements we measured (time effect). These changes were different for the different soil types, as indicated by the significant time*soil interactions. Major changes in concentrations are discussed below. Initial concentrations of all measured elements and ions are given in Table 4.

The artificial river water replaced the original surface water in the aquarium within 2 weeks, as was indicated by the Cl^- concentration (results not shown), which can be used as an inert tracer. Within 1 month, the river water had penetrated all soil types, even the poorly permeable heavy clay soil, as can be seen from the Cl^- concentrations in the pore water (Figure 3a).

The presence of high concentrations of soluble Fe in the pore water of the alder carr and clay soils right from the start of the experiment (Figure 3b) showed that conditions were anoxic from the beginning. Concentrations of Fe in the pore water correlated with those of amorphous iron in the soil determined by oxalate extraction. In the floating fen soil, hardly any iron was present in the

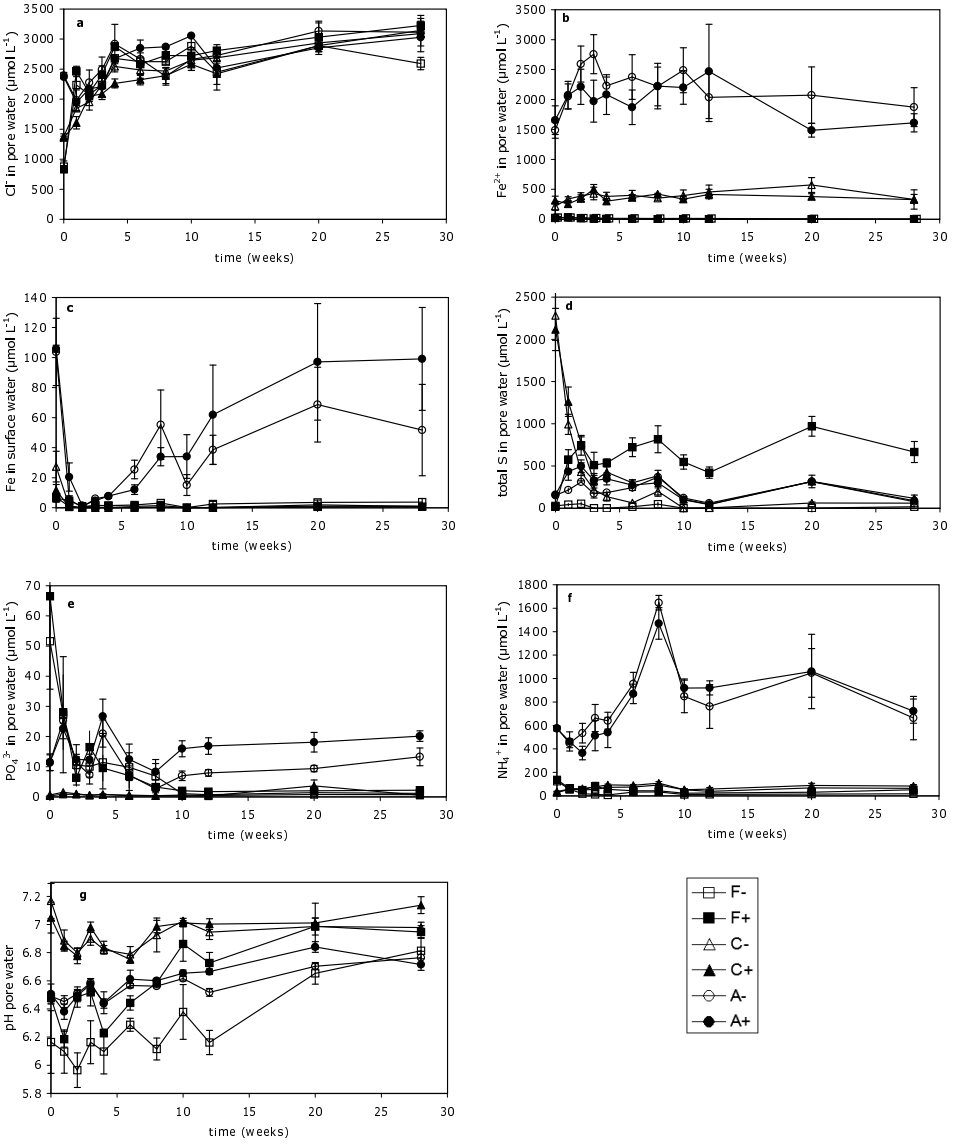

Figure 3. Concentrations of ions in the pore water and the surface water in the aquarium experiments. Open symbols represent treatments without sulphate, closed symbols treatments with added sulphate. Squares: floating fen soil; triangles: clay soil; circles: alder carr soil. Error bars represent standard error of the mean. 3a Concentration of Cl^- in the pore water; 3b concentration of Fe in the pore water; 3c concentration of Fe in the surface water; 3d concentration of total S in the pore water; 3e concentration of PO_4^{3-} in the pore water; 3f concentration of NH_4^+ in the pore water; 3g pH of the pore water.

pore water. In the clay soil, the Fe concentration in the pore water stabilised at 400 µmol L^{-1}. In the alder carr soil, concentrations were extremely high, varying between 1500 and 2500 µmol L^{-1}. Concentrations of Fe in the surface water remained low, except for the alder carr soil (Figure 3c). Only in the iron-poor floating fen soil did sulphate addition lower the availability of iron significantly. At the end of the experiment, iron concentrations in the soil pore water in the floating fen soil were 4.5 µmol L^{-1} on average in the treatment without sulphate and 0.7 µmol L^{-1} in the treatment with sulphate.
Sulphate reduction took place even in the early stages of the experiment. The sulphate treatment caused sulphate concentrations (Figure 3d) in the pore water in the iron-rich soils (C and A) to decrease and remain well below the added concentration of 1000 µmol L^{-1} after one month, even though Cl^- concentrations showed that the sulphate-rich medium had fully penetrated the soil. Initial sulphate concentrations were high for the clay soil, but fell considerably within the first month. The sulphide concentration in the pore water after 28 weeks was below the detection limit for all soils and treatments, except for the sulphate-treated floating fen soil (275 µmol L^{-1}; results not shown).

Starting from week 29, the distance between the floating sods and the bottom of the aquarium became significantly smaller in the sulphate-treated condition (Figures 4 and 5). Sulphate addition had a significant influence on the CH_4 concentrations of the pore water in the floating fen soil. Average pore water concentration in the control was 1700 µmol CH_4 L^{-1} pore water, against 370 µmol L^{-1} for the sunken sods in the sulphate treatment (results not shown).

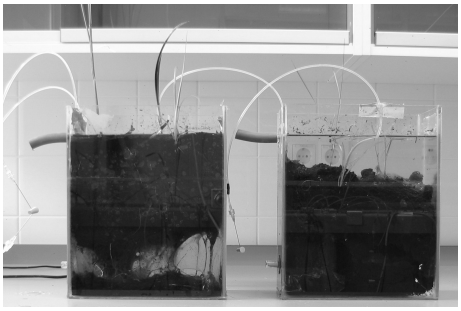

Figure 4. Floating fen soil sinking under the influence of sulphate addition. Control treatment on the left, treatment with sulphate on the right. Also visible are the smaller G. maxima plants in the sulphate treatment.

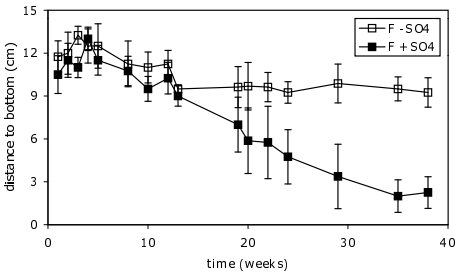

Figure 5. Distance of floating fen rafts from the bottom of the aquaria. F = floating fen, –SO4 = control treatment without sulphate, +SO4 = treatment with sulphate. Error bars represent standard error of the mean.

Initial phosphate concentrations in the pore water were high for the fen soil (Figure 3e), but decreased strongly within the first two weeks. Phosphate concentrations in the surface water of all soils (results not shown) also showed a steep drop at the beginning of the experiment, due to leaching. Concentrations of total P (results not shown) showed the same pattern as phosphate concentrations. Phosphate concentrations in the pore water became high especially in the alder carr soil after 10 weeks (13-20 µmol PO_4^{3-} L^{-1} (Figure 3e), 17-42 µmol total P L^{-1}), compared to the other soil types. Sulphate addition clearly increased the phosphate availability in the pore water of the iron-rich alder carr soil, unlike the other soils. Phosphate concentrations in the pore water of this soil upon treatment with sulphate were significantly higher than in the control (weeks 6-20; about twice as high in week 20). In the surface water, however, this effect of sulphate on phosphate concentrations was only visible as a transient peak in week 20, when surface water concentrations (31 µmol L^{-1}) were 10 times higher in the sulphate-treated condition (results not shown).

NO_3^- was present in very low concentrations from the start of the experiment (results not shown). Within 2 weeks, it dropped below 5 µmol L^{-1} in both the pore water and the surface water, either by reduction under anoxic circumstances, or by leach-out. During the experiment, concentrations of NH_4^+ in the pore water (Figure 3f) were high in the clay and the floating fen soil (10-100 µmol L^{-1}), and extremely high in the alder carr soil (up to 1600 µmol L^{-1}).

As the reduction of Fe^{3+} and that of SO_4^{2-} are acid consuming processes, the pH in the pore water (Figure 3g) rose in all soils during the experiment, the increase being greater for the sulphate treatment (time*treatment interaction). pH also increased in the surface water during the experiment (results not shown). During algal blooms, pH rose above 9 in the surface water of the alder carr soil, as a result of CO_2 depletion by photosynthesis.

Biomass of the phytometers

In the floating fen soil, the addition of sulphate had a negative effect on *G. maxima* biomass production. Here, the addition reduced the biomass by more than 60% compared to the control (results not shown). In the other soils, however, standard deviations of the biomass were too large to identify significant differences between the treatments. This was due to die-off of part of the plants, because they were planted below the water table in these soils. In the floating fen soil, a significant difference in iron concentration in the plant tissue of *L. minor* was found between the treatments (lower in the sulphate treatment), but this did not affect biomass production (results not shown). *G. maxima* did not show lower iron concentrations in the sulphate treatment. Concentrations of other important elements in the phytometer biomass, such as N and P, showed no significant effect of the sulphate treatment.

Table 5. P values of the soil and treatment effects of the CO_2, CH_4 and total C (CO_2 + CH_4) production rates expressed per gram dry weight, as tested by univariate ANOVA (GLM). Bold values indicate P ≤ 0.05.

	CO_2 production rate	CH_4 production rate	Total C production rate
soil	**0.000**	**0.000**	**0.000**
treatment	0.357	**0.021**	0.605
soil*treatment	0.537	0.138	**0.043**

Decomposition experiment

Potential decomposition

Potential decomposition rates (Figure 6, total C) were highest in the floating fen soil. No significant effect of sulphate addition on the potential decomposition rates was found. When corrected for the organic matter content of the soils, decomposition rates in the floating fen soil were four times higher than the rates in the alder carr soil.

Potential production of CH_4

The potential methane production differed considerably between the three soil types. Hardly any methane was produced in the clay soil. In contrast, the floating fen slurry had a very high potential methane production rate (about 9 µmol g^{-1} dry weight day^{-1}; Figure 6), which was 4 times higher than the potential methane production rate in the alder carr slurry. Univariate ANOVA showed that the two organic soils also differed in the duration of the lag phase, i.e. the initial phase in which no methane is produced (4 h for the floating fen soil and

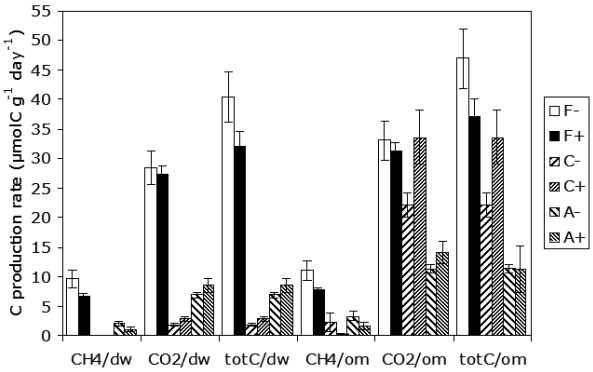

Figure 6. Potential CO_2, CH_4 and total C (CO_2 + CH_4) production rates in the decomposition experiment for the various soils and treatments, expressed per gram dry weight (/dw) as well as per gram organic matter (/om). F = floating fen, C = clay soil, A = alder carr soil. – = control treatment without sulphate, + = treatment with sulphate. Error bars represent standard error of the mean.

215 h for the alder carr soil). A significant effect of the addition of sulphate on the methane production rates was also demonstrated by univariate ANOVA (Table 5), showing the inhibiting effects of sulphate as a more favourable electron acceptor.

Potential production of CO_2

Like that of CH_4, the potential production rate of CO_2 in the floating fen slurry was very high (28 µmol g^{-1} dry weight day^{-1}; Figure 6), four times higher than the potential production rate in the alder carr slurry. In the clay slurry, CO_2 production was measured in the first six days of the experiment, but the production stopped after that time. Although the potential production rate expressed per gram dry weight was 3 times smaller in this clay slurry than in the alder carr slurry, when expressed as potential production rate per gram organic matter, the potential CO_2 production rate in the clay slurry during these six days was even higher than in that of the alder carr.

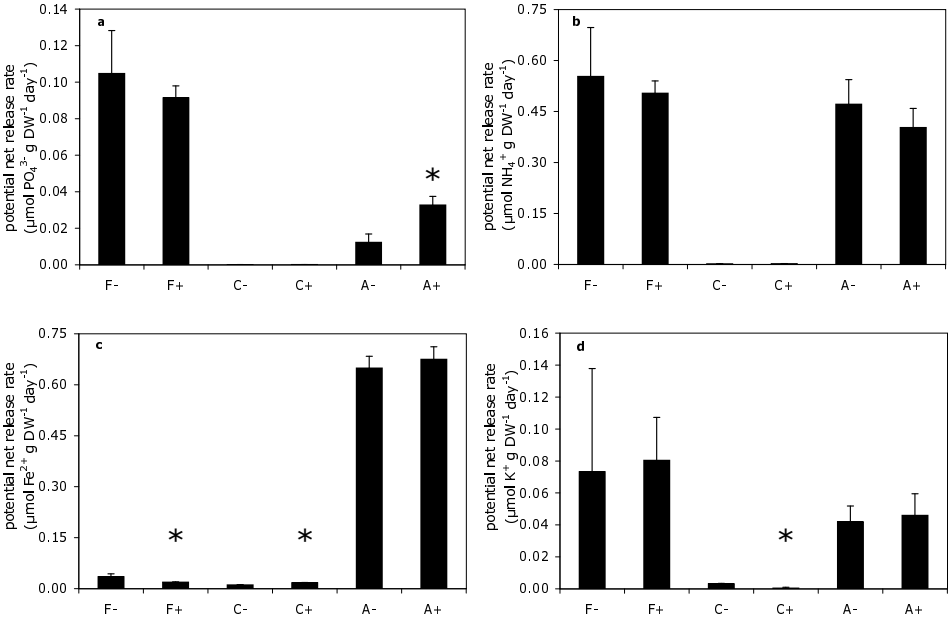

Figure 7. Potential net release rates in the decomposition experiment. F = floating fen, C = clay soil, A = alder carr soil. – = control treatment without sulphate, + = treatment with sulphate. Error bars represent standard error of the mean. 7a Potential net release rate PO_4^{3-}; 7b potential net release rate NH_4^+; 7c potential net release rate K^+; 7d potential net release rate Fe^{2+}. Asterisks mark significant differents between treatments within a soil.

Potential release rates

Figures 7a to 7d show the net potential release rates for PO_4^{3-}, NH_4^+, K^+ and Fe^{2+}. Both of the organic soils (F and A), had high net PO_4^{3-}, NH_4^+ and K^+ release rates. Net Fe^{2+} release was highest in the alder carr slurry, analogous to the aquarium experiment. The ratio between the net release of C and NH_4^+ in the slurry was 3 times higher in the floating fen soil than in the alder carr soil. In the alder carr slurry treated with sulphate, phosphate release was three times higher ($p=0.021$) than in the control. This is comparable to the results of the aquarium experiment. In the iron-poor floating fen soil, the iron release rate was significantly reduced by the addition of sulphate.

Discussion

The three soil types showed large differences in potential anaerobic decomposition rates and release rates. In the clay soil, CO_2 production stopped after six days. The potential decomposition rate per gram of organic matter in these first days was, however, even higher in this soil than in the alder carr soil. This means that during the first six days, easily degradable organic matter was present in the clay soil. Most likely, after a few days the concentration of degradable organic matter had become limiting for decomposition in the clay soil. The potential decomposition rate of the floating fen soil was very high (40 µmol C g^{-1} dw d^{-1}). Maximum values found in the literature vary between 10 µmol C g^{-1} dw d^{-1} for bog peat (Smolders *et al.,* 2002) and 50 µmol C g^{-1} dw d^{-1}, reported for the floating fen in Lake Emlagharan in Ireland (Bontes *et al.,* 2005). This means that the organic matter in the floating fen soil was relatively easily degradable. Potential CH_4 production rates also varied per soil type. Due to the lack of easily decomposable organic material, no CH_4 was produced in the clay soil, i.e. there was a surplus of electron acceptors compared to the organic matter present. This was also the case in the alder carr soil during the lag phase. The high concentration of Fe(III) in this soil inhibited methanogenesis in this phase, because iron-reducing bacteria have a higher affinity to easily degradable organic compounds, such as acetate, than methane-producing bacteria (Roden & Wetzel, 2003). The presence of a lag phase suggests that methanogenesis does not occur in the field, because of the continuous supply of sulphate.

Release rates were high for both of the organic soils. The alder carr soil released more iron, due to its higher amorphous iron concentration. The ratio between the net release of C and N in the alder carr slurry was, however, 4 times lower than in the floating fen soil, although NH_4^+ release rates were equally high: this means that part of the ammonium released in the alder carr soil probably did not originate from the decomposition of organic matter, but

from cation exchange. Cation exchange rates might be higher in the alder carr soil than in the other soils, because of the production of high concentrations of Fe^{2+} by the reduction of Fe^{3+}.

In the aquarium experiment, iron concentrations in the pore water were extremely high in the alder carr soil. Lucassen *et al.* (2000) showed that field concentrations of 2600 µmol Fe^{2+} L^{-1} induce necrotic spots in leaves of *Glyceria fluitans*. Although no such spots were found on *Glyceria maxima* in our experiment, concentrations found in our experiment (up to 2500 µmol Fe L^{-1}) might still be toxic to sensitive plant species in the field. It is known that reduced iron from the soil pore water that diffuses towards the surface water is oxidised and precipitates on the soil-surface water interface due to the higher redox potential of the water column (Cornwell & Kipphut, 1992; Gunnars & Blomqvist, 1997; Moore *et al.,* 1998). This explains the low iron concentrations in the surface water over the clay soil. In the alder carr soil, pore water concentrations were so high that exchange of Fe between the pore water and the surface water took place. Since the surface water was directly connected to the outflow of the aquarium, concentrations in the surface water can be regarded as outflow concentrations. The stability of the pore water iron concentrations and the high weekly iron outflow from the alder carr soil show that iron reduction was still taking place at the end of the experiment. Sulphate reduction rates were also high, indicating simultaneous activity of Fe- and SO_4-reducing micro-organisms (Sørensen, 1982).

Iron reduction played a major role in the mobilisation of phosphate in this experiment. In the alder carr soil, which was rich in iron as well as in total and Olsen-extractable P, continuous iron reduction led to high concentrations of P in the pore water. In this P-rich soil, Fe(III) precipitations at the soil-water interface did not trap all P, and increasing P concentrations were also found in the surface water. In the iron-poor floating fen soil, on the other hand, most of the phosphate release might be attributed to decomposition of organic material. The decomposition experiment showed a high decomposition rate in this soil, as well as high concentrations of phosphate in the slurry at the end of the experiment. There was, however, a striking difference between the high phosphate release in the slurry of the floating fen in the incubation experiment and the low phosphate concentrations in the pore water of the aquarium experiment, whereas the P concentrations in the alder carr were high in both experiments. One of the explanations for this apparent discrepancy might be the difference in phosphate leach-out between these soils. Pore water phosphate concentrations in the floating fen soil were high at the beginning of the aquarium experiment, but decreased very fast after the start of the experiment. Smolders *et al.* (2001) showed that the release of phosphate from the pore water of underwater soils to the water layer strongly depends on the

Fe:PO_4 ratio in the pore water; below a value of 1 (mol mol^{-1}), they measured a strong release of PO_4^{3-} to the surface water. Fe:PO_4 ratios in our experiment were 0.3 (with sulphate) to 5 (without sulphate) for the floating fen soil and 82 (with sulphate) to 176 (without sulphate) for the alder carr soil. This may have caused a higher phosphate release to the water layer from the floating fen soil, and hence a larger leach-out.

Due to the anaerobic conditions, NH_4^+ resulting from the decomposition of organic matter could not be nitrified, and accumulated in the pore water, particularly in the alder carr soil. Another possible source of the high NH_4^+ concentrations in the pore water could be cation exchange of adsorbed NH_4^+ by cations added with the artificial river water (Lucassen *et al.,* 2006), and in the alder carr soil also by the mobilised Fe^{2+} originating from the reduction of Fe(III)oxides. In the alder carr soil, ammonium concentrations rose to 1600 µmol L^{-1}. Lucassen *et al.* (2006) showed that the presence of target vegetation in alder carrs is correlated with low concentrations of ammonium (<200 µmol L^{-1}). Hence, the concentrations measured in this experiment, if present in the field, could seriously hamper the intended vegetation development.

The response of the different soils used in this study to inundation with sulphate-rich river water in the aquarium experiment varied substantially, reflecting their soil characteristics. Sulphide, produced by reduction of the added sulphate, was present in excess relative to iron in the floating fen soil. Hence, the binding of sulphide to iron reduced the availability of soluble iron in this soil, both in the aquarium and in the incubation experiment. As iron concentrations were low, phosphate release as a result of the binding of iron to sulphide was equally low. Part of the sulphide produced could not be bound to iron and was freely available in the pore water in toxic concentrations, hampering the growth of the planted *Glyceria maxima*. The sulphide concentrations we measured in the pore water have been shown to be toxic to several wetland plants (Smolders & Roelofs, 1996; Van der Welle *et al.,* 2006; 2007). Moreover, the addition of sulphate decreased the methane concentration in the floating fen soil in the aquarium experiment and the potential methane production rate in the incubation experiment. Methane concentrations determined the buoyancy of the floating fen, just as had been demonstrated earlier for other floating fens and peat bog rafts (Hogg & Wein, 1988a; Swarzenski *et al.,* 1991; Sasser *et al.,* 1996; Lamers *et al.,* 1999; Smolders *et al.,* 2002). The floating fen soil sank under the influence of sulphate as a result of methanogenic bacteria being outcompeted by sulphate-reducing bacteria (Cappenberg, 1974; Abram & Nedwell, 1978; Lovley & Klug, 1983; Westermann & Ahring, 1987; Prieme 1994; Scholten & Stams, 1995), in the same way as this is known to happen in floating peat bogs (Lamers *et al.,* 1999; Smolders *et al.,* 2002). In the other soils, sulphide formed by the reduction of sulphate was bound to the excess

iron. In both the aquarium experiment and the incubation experiment, this led to an additional release of phosphate in the alder carr soil. Sulphate addition did not increase the decomposition rates, as had been found earlier in bog peat (Lamers *et al.,* 1999; Smolders *et al.,* 2002; Tomassen *et al.,* 2003). In soils where sulphate increases decomposition, this seems to be an effect of the increased alkalinity produced by the reduction of sulphate (Lamers *et al.,* 1999; Smolders *et al.,* 2002; Tomassen *et al.,* 2003). In our experiment, alkalinity of the artificial river water was already high (2000 µmol HCO_3^- L^{-1}, similar to concentrations in the water of the river Meuse). Since no effect of sulphate on the decomposition rate was found, it is likely that the additional release of phosphate in the sulphate treatment of the alder carr soil in the incubation experiment did not originate from decomposition of organic matter, but can be attributed to the competition between sulphide and phosphate for binding to iron.

Our findings show the importance of both soil type and water quality in the biogeochemical response to flooding. Inundation has the smallest impact on soils without organic matter, in terms of phosphate release by reduction of iron(III) and the formation of potentially phytotoxic reduced compounds such as ammonium, iron(II) and sulphide ('internal toxification'). Flooding of soils containing organic matter, in contrast, leads to accumulation of ammonium and release of Fe-bound phosphate. This internal eutrophication could cause algal blooms in the water layer above such soils. Additionally, river water polluted with sulphate may cause sulphide phytotoxicity if the iron content is low, as was shown here for the floating fen soil. If the soil is richer in iron, sulphide will be bound to iron and may release phosphate that was bound to the iron, as took place in the alder carr soil. It should be noted that these two processes, sulphide toxicity and enhanced phosphate mobilisation, can also take place in the same soil, as was found for instance by Lamers *et al.* (1998) for a mesotrophic fen.

The presence of sulphate reduces methanogenesis, which is a very important process in ecosystems depending on methane for floating, such as floating fen mats and bog peat rafts. Floating fen mats form the first stage in the terrestrialisation process in fen systems. Our results show that inundation of these floating fens with sulphate-rich river water at levels occurring in the rivers Meuse and Rhine may thus destroy the floating fens and their rare vegetation as well as block the subsequent terrestrialisation process and the accompanying vegetation succession. Analogously, we expect the elevated nitrate concentrations in the river water to have a similar inhibiting effect on methanogenesis (Westermann & Ahring, 1987; Scholten & Stams, 1995) and on the buoyancy of floating fens.

The results presented in this article have important implications for the rehabilitation and creation of wetlands, and for the selection of locations for the creation of flood water storage areas. Completely mineral soils, such as those in clay and sand sedimentation areas where the topsoil has been removed, are not likely to be at great risk of eutrophication in the short term. However, wetland locations containing organic matter – and most soils do so – should be chosen with greater care. Here, other soil characteristics such as iron and phosphate concentrations, and water quality parameters such as sulphate concentrations, should be taken into account.

Acknowledgements

We would like to thank Martin Versteeg for his help in collecting the soils, Roy Peters and Germa Verheggen for their technical support, and Jelle Eygensteyn for his help with the analyses. We thank Jan Klerkx for improving the English of the text. This study was financially supported by the Netherlands Organisation for Scientific Research (NWO) as a part of the TRIAS programme (project 835.80.010).

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4

Prediction of phosphorus mobilisation in inundated floodplain soils

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Environmental Pollution, in press

Abstract

After flooding, iron reduction in riverine wetlands may cause the release of large quantities of phosphorus. As phosphorus is an important nutrient causing eutrophication in aquatic systems, it is important to have a tool to predict this potential release. In this study we examined the P release to the soil pore water in soil cores from floodplains in the Netherlands and from less anthropogenically influenced floodplains from Poland. During the inundation experiment, concentrations of P in the pore water rose to 2–90 times the initial concentrations. P release was not directly related to the geographic origin of the soils. An important predictor variable of P release was found in the ratio between the concentration of iron-bound P and amorphous iron. This ratio may provide a practical tool for the selection of new areas for wetland creation, and for impact assessment of plans for riverine wetland restoration and flood water storage.

Mobilisation of phosphorus in floodplain wetland soils can be predicted with easily measurable soil characteristics.

Introduction

In recent decades, much attention has been devoted to the creation and rehabilitation of wetlands in riverine areas. Under the Ramsar Convention and the European Union Habitats Directive, many countries have designated wetlands in estuaries and riverine areas for conservation. Additionally, certain areas within floodplains have been earmarked to be used for temporary water storage in times of high river discharges to prevent floods in densely populated areas (e.g. the "Space for the River" policy in the Netherlands). Rehabilitation of riverine marshlands has been found to be important for both floristic and faunistic biodiversity (Nienhuis *et al.*, 2002), and recently excavated permanent water bodies, such as side-channels and small lakes, are important for waterfowl. Many of the soils of these new conservation areas, however, contain high nutrient concentrations originating from former agricultural use or from river water pollution in the past (Lamers *et al.*, 2006).

Phosphate generally limits primary production in aquatic systems (Schindler, 1977; Reddy, 1999). Inorganic P in wetland soils is predominantly present in the form of precipitates with calcium (calcium phosphates and P included in/adsorbed to calcium carbonates), or adsorbed onto aluminium and iron oxides and hydroxides, and precipitated as vivianite (Goldberg & Sposito, 1984; Golterman, 1995). Iron oxides and hydroxides are susceptible to redox changes. Upon inundation, sediment can become anoxic in a short time, as oxygen trapped in the soil is used up by micro-organisms for aerobic decomposition of organic matter, and diffusion from the overlying flood water to the sediment is very slow. Under anaerobic circumstances, NO_3^-, Mn^{4+}, Fe^{3+} and SO_4^{2-} are used as alternative electron acceptors for microbial decomposition. The reduction of Fe(III) in lakes has long been known to be accompanied by a release of P (Einsele, 1938; Mortimer, 1941, 1942; Patrick & Khalid, 1974). The concurrent release of Fe and P has also been observed in the shallow groundwater of river riparian zones at low dissolved oxygen concentrations (Carlyle & Hill, 2001). It is generally assumed that this concurrent release of P and Fe is caused by the reduction of Fe(III) oxides and hydroxides, mobilizing Fe (II) and releasing part of the adsorbed P, as the affinity of P to Fe(III) is higher than to Fe(II). Additionally, release by bacteria, induced by the switch from aerobic to the slower anaerobic decomposition and occurring at the same time as iron reduction, may also play a role in the release of P upon flooding (Gächter & Meyer, 1988; Baldwin & Mitchell, 2000).

Floodplains of lowland rivers are usually seen as net sinks for nutrients because of sedimentation (Van den Brink *et al.*, 1993; Van der Lee *et al.*, 2004; Olde Venterink *et al.*, 2003). Since riparian wetlands have much higher pore water P concentrations under anoxic circumstances than the oxic river water, however,

they can periodically function as a source of P to the river water (Carlyle & Hill, 2001). Because of the agricultural origin of most of the newly created nature reserves within the Dutch floodplains, it is possible that inundation, especially in warmer periods, leads to large-scale phosphate mobilisation and to leaching of phosphate into the river water or into the surface water of new lakes and water retention areas. This may ultimately lead to eutrophication and, in stagnant water, to algal blooms. The mobilisation of P to the pore water as such may also increase direct P availability for rooting riparian plant species.

Most studies have tended to describe phosphate retention and release by phosphate sorption characteristics in equilibrium situations (e.g. Khalid *et al.* (1977), Boström *et al.* (1988), Reddy *et al.* (1999) and Bridgham *et al.* (2001)). During flooding, however, equilibrium between oxidised and reduced iron species in soil pore water is generally not attained. Under these circumstances, predicting the kinetics of phosphate release may give better results than using equilibrium isotherms. In this study, we aimed to find simple soil characteristics that could predict the P mobilisation to the soil pore water due to iron reduction. Furthermore, as we expected soils from less polluted areas to release less phosphate, we compared soils from floodplains along regulated Dutch rivers with soils from the more pristine river Narew in the east of Poland.

Materials and methods

Soil cores 12 cm in diameter and approximately 15 cm in depth were taken from four different floodplains in the Netherlands and one in Poland (Figure 1). All soils were fluvisols according to the FAO/Unesco classification (Driessen & Dudal, 1991). They were collected in triplicate from a total of nine different vegetation types. Additionally, three soil samples, consisting of at least five subsamples with a depth of about 15 cm, were taken from an area of 20 m^2 with an auger at each location. These soil samples were stored in polyethylene bags at 4°C until further analysis.

Site descriptions

Huis den Doorn (N 52°33'22", E 06°07'16") is a nature reserve along the Dutch small river Overijsselse Vecht. The floodplain in which it is situated is flooded at least once a year for several days in late winter. The field where the cores were taken was used as a meadow in the past and is still mown once a year. It has a patchy vegetation structure. We took our samples from sites dominated by *Carex acuta* (soil A) and by *Glyceria maxima* (soil B). The soil is made up of sand and peat on a clay subsoil.

At the town of Heinenoord (N 51°49'49", E 04°30'46"), the river Oude Maas, which is a branch of the Rhine/Meuse estuary, is a freshwater tidal river, whose floodplains are flooded twice a day. Soil cores were taken from a reed marsh (soil C), which also supported tall herbs, and from the adjacent low-lying mud flat, which was sparsely vegetated by *Scirpus lacustris* (soil D). Both soils consisted of sands, mixed with 5-10 % organic matter.

The Koningssteen (N51°08'56", E 05°50'55") nature reserve is situated on the shore of a lake directly connected to the river Meuse. The site from which the soil cores were taken is separated from the lake by a small bank protection, which shelters the shores from the surge of waves. This bank protection has allowed a thin organic layer of approximately 5 cm, to be formed on top of the sandy soil. The vegetation of this site is dominated by *Mentha aquatica* and *Leersia oryzoides* (soil E). The reserve is flooded in winter, but the shores of the lake remain wet or moist all year round. The area is grazed by cattle.
The Steenwaard nature reserve (N 51°57'55", E 05°12'18") is a fairly recently created reserve along the river Rhine, flooded several times per winter. Our sites have soils consisting of heavy clay. On soil F, *Plantago major* was the dominant plant species. This site is heavily grazed by geese in winter. Soil G is lower-lying and hence wet for most of the year due to stagnating rain and river water. One of the dominating plant species here is *Bidens cernua*.

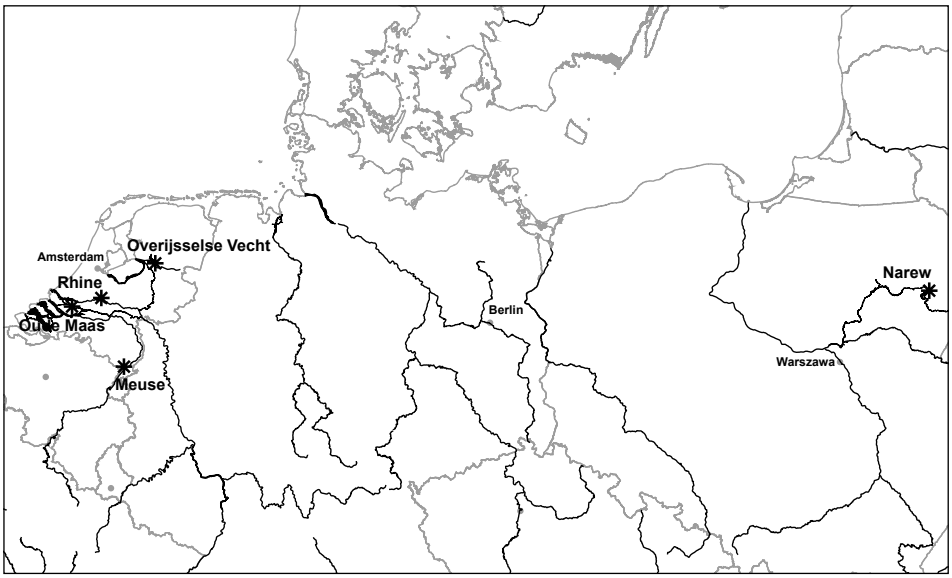

Figure 1. Location of the sampling sites. Samples were taken from the rivers Overijsselse Vecht (soils A and B), Oude Maas (soils C and D), Meuse (soil E), Rhine (soils F and G) and Narew (soils H and I).

In the east of Poland (N 53°08'10", E 22°09'35") we took soil cores from a floodplain along the river Narew. Soil H is taken from a dry sandy grassland, extensively grazed by cattle, and is flooded only at high river discharges. It has a high botanical diversity, in which small sedge species and grass species such as *Phalaris arundinacea* are abundant. A few hundred metres from the site of soil H, there is a peaty oxbow lake, featuring *Stratiotes aloides* mats. On its shores, composed of sand and organic matter (14%), soil cores were taken from a vegetation dominated by *Agrostis stolonifera* (soil I).

Experimental set-up

In the field, soil cores were transferred to plastic containers of the same inner size, leaving the original soil structure intact. They were placed in climate chambers at a 20˚C/12h and 15˚C/12h temperature regime to mimic summer field conditions. Aboveground biomass was carefully removed and the cores were covered with black plastic to prevent algal growth. Cores were inundated with artificial river water (Na 2000, Ca 1300, Mg 250, K 250, Cl 3314, HCO_3 2000, NO_3 35 and HPO_4 0.3 µmol L^{-1}), which had the average composition of the river water at the Dutch sites, to approximately 3 cm above the soil surface. Two rhizon samplers (Rhizon SMS -10cm, Rhizosphere Research Products) were placed in each soil core. Just after the start of the experiment, and subsequently on a regular basis for six months (see Figure 3 for times of sampling), soil pore water samples (approximately 100 mL per sample) were taken from the cores using vacuumed glass bottles. Water losses were compensated with artificial river water.

Chemical analyses

Immediately after collection of the pore water, the concentration of total dissolved sulphide species was determined in a 10 mL subsample, fixed with S^{2-} antioxidant buffer containing NaOH, NaEDTA and ascorbic acid. S^{2-} was detected using an S^{2-} ion-specific Ag electrode and a double junction calomel reference electrode (Thermo Orion) (Van Gemerden, 1984). Total S was regarded as SO_4, as, at the concentrations occurring in our samples, only a very small part will be in organic form and no free S^{2-} was found to be present. Pore water samples and soil extracts were analysed for total P, Fe, Mn, Ca and S by ICP-OES (ICP, Spectroflame VML2). For the ICP analyses, concentrated nitric acid was added up to a concentration of 1.17% to the pore water and extraction samples. Samples were stored in polypropylene tubes at 4˚C. For the other analyses, 0.12 g citric acid L^{-1} was added to the samples. They were stored in iodated polyethylene bottles at -24˚C until further analysis. Ammonium

(NH_4^+) and nitrate (NO_3^-) (+nitrite (NO_2^-)) were analysed colorimetrically using ammonium molybdate, ferriammonium sulphate, salicylate and hydrazine sulphate, respectively (TRAACS 800+ AutoAnalyser, Bran+Luebbe, Norderstedt, Germany). Pore water pH was measured with a Radiometer Copenhagen type PHM 82 standard pH meter.

To determine the different pools of P, a series of sequential P fractionations after Golterman (1996) were performed in duplicate on one of the composite soil samples from each location. The advantage of this method compared to more commonly used P fractionation methods (e.g. Hieltjes & Lijklema (1980)) using NaOH as an Fe-P and Al-P extracting step, is that this method does not extract humic and fulvic acids in this step. Two grams of field-moist soil were shaken twice (100 rpm) for 2 h with 25 mL 1M NH_4Cl to extract loosely bound and water-soluble phosphorus. The pellet was then shaken twice for 2 h with 30 mL 0.05 M Ca-EDTA to extract Fe- and Al-bound P (referred to below as Fe-bound P (Fe-P)). Subsequently, the pellet was extracted twice with 30 mL 0.1 M Na-EDTA for 18h to extract Ca-bound P. The remaining pellet was digested with HNO_3 and H_2O_2 as described below. The phosphorus dissolved in this step was considered to be organic P. The efficiency of this P fractionation method was checked by comparing the sum of the extracted P in the different steps with the total concentration of P as determined by direct digestion of the soil samples instead of the pellet. The total concentration of P in the sediments was 32.0 µmol g DW^{-1} on average, with a mean difference of 2.2 µmol g DW^{-1} caused by the extraction methods.

Organic matter content of the sediments was determined by loss-on-ignition (4h, 550˚C). In order to determine the total concentrations of elements in soil material, 200 mg of homogenized and dried sample was digested for 17 minutes, heated by microwave, with 4 mL concentrated HNO_3 and 1 mL 30% H_2O_2 (Milestone microwave MLS 1200 Mega). Soil extractions were performed on fresh sediments to avoid changes in the distribution of P and Fe over the different pools, and were corrected for moisture content afterwards, after drying (24h, 105˚C). The concentration of amorphous Fe was determined by oxalate extraction (2.5 g of sediment shaken for 2h with 30 mL of a solution containing 16.2 g of $(COONH_4)_2.H_2O$ and 10.9 g of $(COOH_2).2H_2O$ per L) (Schwertmann, 1964).

Statistical analysis

All statistics were calculated with SPSS 14.0 (SPSS Inc., 2005), using the means of the pore water analyses of the three soil core replicates of the correlation and regression calculations. Normality of distributions was tested by a Kolmogorov-Smirnov test. A t-test was used to test differences in concentra-

tions of total P, and Fe-P in soil and the final concentrations of P in the pore water between the Dutch and Polish soils. Correlations were described by Pearson correlation, or by Kendall's tau correlation for the factors that did not meet the assumption of a normal distribution. Linear regression was used to estimate pore water P concentrations using select soil characteristics as independent variables. Significance was accepted at a probability level of $P \leq 0.05$.

Results and discussion

Soil characteristics

All soils could be characterized as mineral soils, varying in organic matter content between 5 and 19% (Table 1). Total Fe concentration of the soils ranged from 72 µmol Fe g^{-1} dw to 1129 µmol Fe g^{-1} dw. Amorphous iron concentrations differed by a factor of more than 25 among the various soils. Soil I had the lowest amorphous Fe concentration (22 µmol Fe g^{-1} dw), while soil A had the highest concentration (600 µmol Fe g^{-1} dw). The percentage of amorphous Fe ranged from 9% to 85% of the total Fe concentration in the different soils. Concentrations of total P also differed considerably among the soils, ranging from 18 µmol P g^{-1} dw to 82. Concentrations of total P in the Polish soils did not differ significantly ($p = 0.258$) from those in the more polluted Dutch soils, but were in the lower part of the concentration range.

The distribution of P among the different pools varied across samples (Figure 2). Concentrations of the NH_4Cl-extractable P pool (less than 3% of the total P), were very low in all soils (less than 0.8 µmol P g^{-1} dw). A larger proportion of the total amount of P in the soil consisted of Fe- and Al-bound P (referred

Table 1. Main soil characteristics derived from digestions and organic matter concentration (OM) derived from loss-on-ignition, pH and Ca concentrations in the soil pore water at the beginning and P concentrations at the end of the inundation experiment. Standard error of the mean in brackets. Am. Fe = Amorphous-Fe; * in triplicate; ** in duplicate.

Soil	OM* (g kg^{-1})	Total Fe* (µmol g^{-1})	Am. Fe* (µmol g^{-1})	Total P* (µmol g^{-1})	Fe-P** (µmol g^{-1})	pH start*	[Ca] start* (µmol L^{-1})	[P] end* (µmol L^{-1})
A	190 (20)	1129 (51)	600 (88)	82 (18)	17 (7)	6.8 (0.2)	1014 (95)	46 (9)
B	70 (0.9)	72 (8)	62 (12)	27 (2)	10 (1)	5.9 (0.2)	1432 (283)	153 (11)
C	100 (30)	180 (29)	85 (23)	20 (2)	4 (1)	7.2 (0.4)	3870 (2410)	20 (2)
D	50 (7)	152 (15)	58 (5)	23 (3)	7 (1)	7.0 (0.03)	2701 (60)	77 (22)
E	50 (7)	303 (30)	110 (6)	19 (0.1)	9 (2)	7.0 (0.3)	3216 (164)	44 (25)
F	70 (3)	300 (11)	71 (9)	30 (0.6)	11 (0.03)	7.3 (0.06)	4501 (1895)	108 (46)
G	60 (3)	357 (42)	64 (21)	31 (0.7)	8 (2)	7.2 (0.1)	3428 (950)	71 (39)
H	140 (7)	114 (3)	54 (6)	18 (0.4)	0.4 (0.2)	6.0 (0.3)	1787 (233)	61 (14)
I	160 (4)	240 (5)	22 (2)	28 (81)	6 (0.7)	7.1 (0.3)	7950 (698)	208 (62)

to below as Fe-bound P). Concentrations in this pool varied between 1 and 47% (Table 1). Soil A had an Fe-bound P concentration of 17 µmol P g^{-1} dw, whereas soil H had a concentration of only 0.4 µmol P g^{-1} dw. The soils from the generally less fertilised sites from Poland contained significantly less iron-bound P than the Dutch soils (3 versus 9 µmol P g^{-1} dw, $p = 0.021$), although ranges showed some overlap. Calcium-bound P also constituted an important proportion of the P in the soil. Concentrations were the highest in soil A (46 µmol P g^{-1} dw), and varied between 2 and 12 µmol P g^{-1} dw for the other soils. Notwithstanding the low organic matter contents of the soils, the organic matter formed an important pool of P (22 to 83% of the total P). Pore water of all soils had a circumneutral pH and ranged from from 5.9 to 7.3. Inspite of the fact the the soils were not calcareous, concentrations of Ca in the pore water were rather high, varying from 1000 upto 8000 µmol L^{-1}.

Effects of flooding

Although we expected the concentration of P in the soil pore water at the end of the experiment (Table 1) to depend on the geographical origin of the samples, this was not the case. The samples from the less anthropogenically influenced river Narew were in the lower part of the concentration range of total P in the soil, as well as in that of Fe-P. The final concentrations of P in the

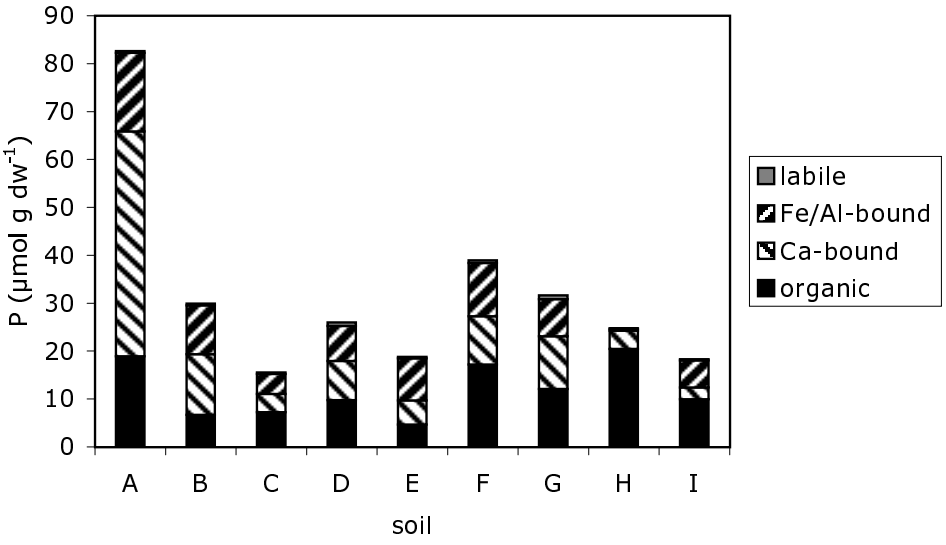

Figure 2. Concentrations of different P pools in the soils according to P fractionation.

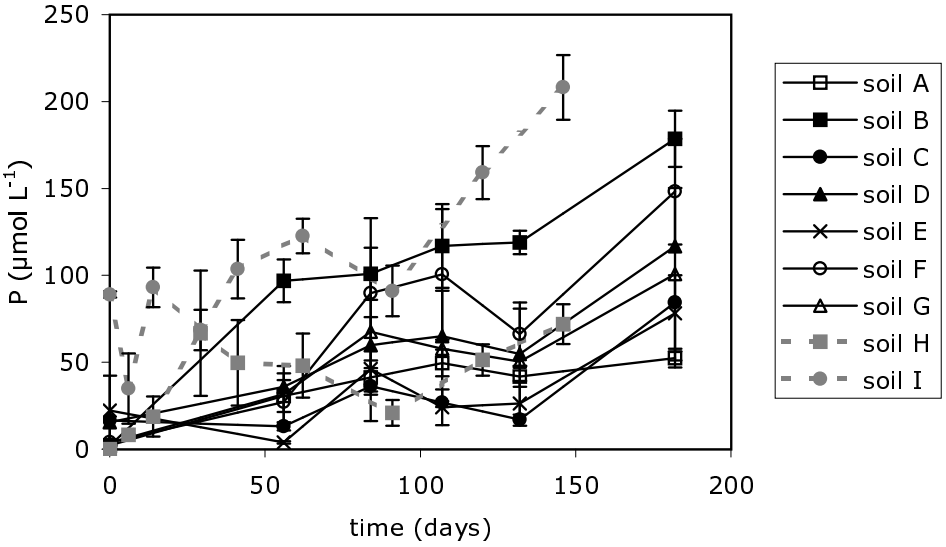

Figure 3. Concentrations of P in the soil pore water over time in the various soils. Error bars represent the standard error of the mean.

soil pore water, however, did not differ from (soil H) or were even higher than (soil I) those in the soils from the more polluted Dutch sites.

Concentrations of total P (hereafter referred to as P) increased in the pore water of all soils (Figure 3). Pore water concentrations at the end of the experiment were 2 (soil I) to 90 (soil B) times those at the beginning of the experiment. Apart from soil I, the increase was smallest in soils that were originally waterlogged in the field situation (soils C, D, E and H). The increase of P in the pore water was accompanied by changes indicating a decrease in redox potential. Nitrate and sulphate disappeared (results not shown), as they were used as electron acceptors. Manganese and iron increased in the soil pore water, as they were mobilised by the reduction of Fe(III) and Mn(IV). Ammonium concentrations increased, indicating limits on nitrification due to the anoxic environment. Due to the inundation with the artificial river water, calcium concentrations in the pore water converged towards the concentrations in the artificial river water (results not shown). Pore water pH values (results not shown) remained the same, or increased in the soils with the lowest pH due to acid consumption in the anoxic reduction processes.

Prediction of phosphorus mobilisation to the pore water

We expected P release upon flooding to be dependent on the concentration of Fe-bound P, since this P pool is the main source from which P is released to the pore water once the soil has become reduced. As can be seen from Figure 4a , there was no correlation between the concentration of Fe-bound P in soil and the P concentrations at the end of the experiment (r = 0, p = 1.00). The total P concentration in the soil could not be used to predict the P concentration in the pore water either (Figure 4b, τ = 0.167, n.s.). These results did, however, not come as a surprise, because we expected P release to depend on the reduction of Fe(OOH). Therefore concentrations of P in the pore water will probably only depend on the initial concentration of Fe-bound P when all Fe in the soil has been reduced. In temporarily flooded iron-rich soils, however, the duration of flood events is usually too short to reduce all Fe.

Of course, part of the P release may originate from mineralisation of organic matter, especially during such long inundation as we used in our experiment. We did not, however, find a significant relationship between the organic matter content and the P release in our soils (r = 0.15, p = 0.700). Phosphorus can also be released when Ca complexes in soil are being dissolved, which highly depends on pH changes. However, significant correlations with the concentration of P at the end of the experiment were found neither for the pH at the end of the experiment (r = 0.192 , p = 0.621), nor for the concentration of calcium at the end of the experiment (r = -0.016, p = 0.968).

The release of P due to the reduction of iron will depend on both the Fe reduction rate and the saturation of Fe with P. As can be seen from Figure 4c, the ratio between Fe bound P to amorphous Fe correlates very well with the concentration of P in the pore water at the end of the experiment (r = 0.887, p = 0.001). The R^2 of this relationship is 0.787, indicating that about 79% of the variation in P concentration in the pore water at the end of the experiment could be explained by the ratio between iron bound P and oxalate extractable iron in soil, underlining the fact that the reduction of iron is the most important process determining P release in these floodplain soils. In this ratio iron reduction rates have, however, not been taken into account. These rates are not easily measurable, as the release of Fe to the pore water is not a reliable measure for the reduction rate. Part of the iron will be reoxidised to Fe (III), and disappear from the pore water (Boström *et al.*, 1988; Cornwell & Kipphut, 1992; Gunnars & Blomqvist, 1997; Moore *et al.*, 1998). Fortunately, the relationship between the P concentration and the ratio of Fe-P to amorphous Fe is a good predictor even without knowing the iron reduction rates. However, as iron reduction rates in inundated soils may differ widely on other factors such as on temperature an the degradability of organic matter in soil, this ratio can only

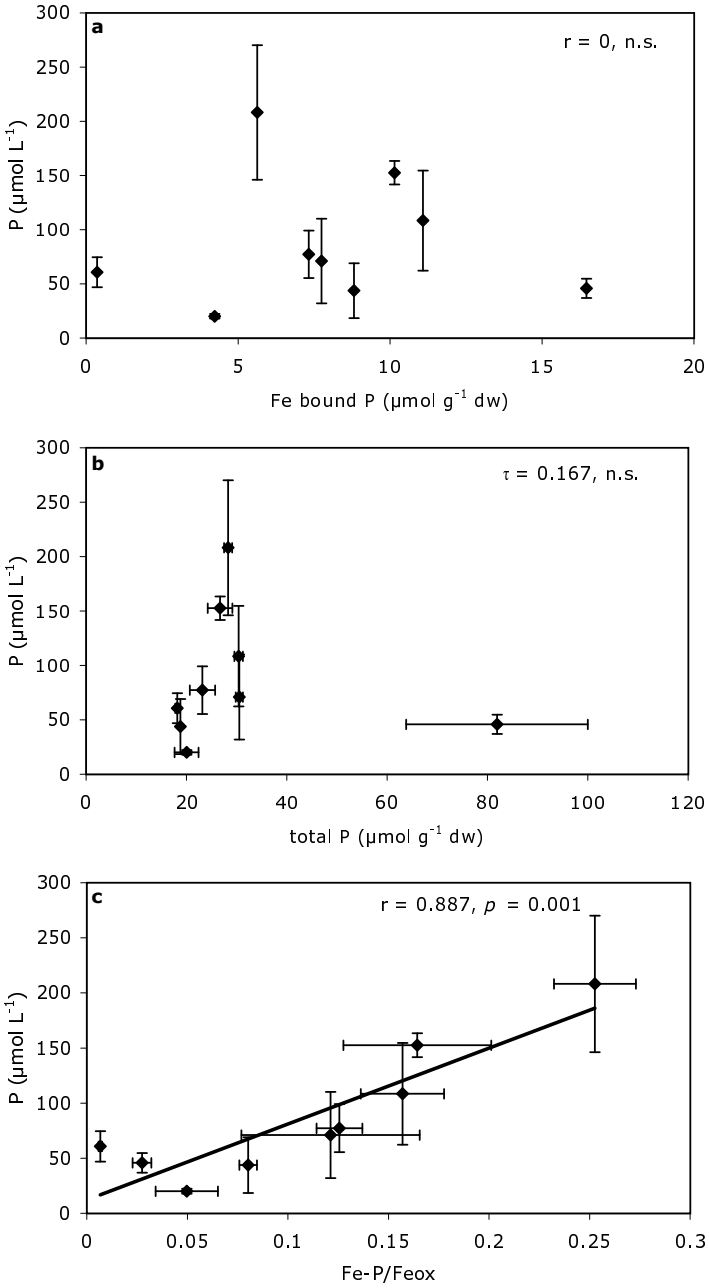

Figure 4a-c. P concentrations at the end of the inundation experiment (µmol L-1) plotted against Fe-bound P (Figure 4a), total P (Figure 4b), and the ratio between Fe-bound P and amorphous iron (Figure 4c). Error bars represent the standard error of the mean. Lines represent regression lines.

be used in a comparative way and not for the exact prediction of the amount of P that will be released to the pore water upon flooding. Young & Ross (2001), studying flooded soil microcosms from floodplains, found a good relationship between the logarithm of the pore water P and the logarithm of the degree of P saturation, being the ratio between the logarithms of the iron-bound and aluminium-bound P and the P sorption index (related to the Langmuir sorption maximum). It can be argued that the relationships we found are roughly the same, as the concentration of amorphous iron in a soil determines the sorption maximum.

An additional release of P to the pore water could also be induced by the formation of sulphides. As the affinity to iron is higher for sulphide than for phosphate, the latter can be released during the precipitation of iron with sulphide (Caraco *et al.*, 1989; Roden & Edmonds 1997; Lamers *et al.*, 1998, Loeb *et al.*, 2007). If this were an important process in a soil, as can be expected under the influence of sulphate-rich water, the relationship described above would be completely different.

Although the release of P into the pore water is the initial step, the release of P from the soil pore water to the river water is decisive for the eutrophication of surface waters and the discharge of P by river water. Since the sediment-water interface often also forms the interface between the anaerobic and aerobic environments, and the top layer of the sediment may also be oxic, the release of P to the water layer cannot be directly predicted by the release of P to the pore water. Reoxidised Fe adsorbs phosphates diffusing towards the surface water and in this way prevents release to the water layer (Boström *et al.*, 1988; Moore & Reddy, 1994; Cornwell & Kipphut, 1992; Gunnars & Blomqvist, 1997; Moore *et al.*, 1998). Release of P to the surface water has been reported to be determined by a number of factors, including the Fe:P ratio in the pore water (Smolders *et al.*, 2001), the Fe:P ratio in the aerobic top layer of the sediment (Jensen *et al.*, 1992), wind and bioturbation, and precipitation with $CaCO_3$ which increases as the pH of the sediment rises because of reduction processes (Boström *et al.*, 1988). On the other hand, a microcosm experiment with seasonally flooded soils by Young & Ross (2001) showed a very close correlation between floodwater phosphate and the phosphate concentration in the pore water itself. In this case the release of P to the surface water may also correlate with ratio between the iron bound P to amorphous iron.

Conclusions

We found the relationship between iron-bound P and the amount of amorphous Fe to be a strong predictor of the extent of P release to pore water in floodplain

soils during flooding and stagnation of surface water. Since the iron reduction rate is largely determined by microbial activity and therefore depends on the temperature, it will not be possible to use this ratio to predict the exact quantitative release in the field. Additionally, soils containing much organic matter, or soils under the influence of sulphate enrichment, may release more P than would be estimated on the basis of the relationship we found. On the other hand, only a small part of the iron is reduced in soils containing very little degradable organic matter, and the release of P may then be overestimated. Nevertheless, we showed that this ratio, which is based on simply measurable soil characteristics, may provide a useful and practical tool for the relative comparison among soils in the assesment of eutrophication risks related to flooding. This tool could hence be used in the designation of flood water storage areas and assessments of the consequences of riverine wetland restoration.

Acknowledgements

We would like to thank Etienne van Boxsel, Denise Buijk, Kim Kobes, Jeroen Geurts, Germa Verheggen, Roy Peters, Jelle Eygensteyn, Martin Versteeg and Liesbeth Pierson for their help with sample collection and analyses. We also want to thank the Dutch State Forestry Service (SBB), Łomzynski Park Krajobrazowy Doliny Narwi, Vereniging tot Behoud van Natuurmonumenten and Stichting het Utrechts Landschap for their kind permission to collect the soil cores and samples. We thank Jan Klerkx for linguistic advise. This study was financially supported by the Netherlands Organisation for Scientific Research (NWO) as a part of the TRIAS program (project 835.80.010).

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5

Effects of restored salinity and tidal regime on biogeochemical processes and vegetation in the Rhine-Meuse estuary; a mesocosm experiment

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Submitted

Abstract

Estuaries are among the most threatened wetlands worldwide. In 1970, most of the Rhine and Meuse estuary was closed off from the open sea by the Haringvliet sluice dam and changed into a stagnant freshwater basin. Currently, authorities are supporting the intention to partly reopen the sluices to regain part of the lost biodiversity. In this study, we examined the effects of the increased tidal amplitude and salinity associated with a partial reopening of the sluices on soil biogeochemistry and vegetation. Effects were studied in mesocosms using sods from a nature reserve and an agricultural area on the banks of the Haringvliet estuary. In a full factorial design, the mesocosms were exposed to a tidal amplitude of 0.2 m and a salinity of 3 ‰ (oligohaline) for one year. Pore water and surface water analyses revealed that the tidal regime enabled oxygen penetration into the soil, decreasing anaerobic processes including iron reduction, leading to an overall immobilisation of phosphate. The tides also decreased methanogenesis. The intrusion of oligohaline water led to increased cation exchange, resulting in higher concentrations of calcium in the pore water and leaching of this ion into the surface water. In the brackish treatments, biomass production was hampered by the increased salinity. Higher concentrations of asparagine, proline and serine in leaf tissue of *Agrostis stolonifera* also indicated increased salt stress in the brackish treatments. As a result, plant species adapted to oligohaline water are expected to show increased competitive strength, enabling the restoration of characteristic vegetation types in the field.

Introduction

Worldwide, coastal ecosystems including estuaries have been strongly modified by human intervention for economic and safety reasons (Kennish, 2002). The Rhine-Meuse delta is a striking example of such a heavily altered estuarine system, in which both tidal movements and salinity gradients have been strongly affected (Smits *et al.,* 2006). In the wake of the major flooding disaster in 1953, this estuary, the Haringvliet, was closed off in 1970 by means of a dam, which prevents the intrusion of sea water into the river and is only opened during low tide to discharge river water (Smits *et al.,* 2006). After the closing of the sluices, the water in the Haringvliet became fresh and tidal amplitude decreased from 2 m to 0.3 m (Bol & Kraak, 1998; Paalvast *et al.,* 1998). Before the sluices were closed, salinity ranged from 17‰ Cl on the seaward side to 0.3‰ Cl at distances of 15 – 25 km inland (Bol & Kraak, 1998). The closing of the sluices reduced the area of intertidal habitats in the Haringvliet by 97%, and that in the upstream Bieschbosch freshwater nature reserve by 93% (Paalvast *et al.,* 1998). As a result of these environmental changes, characteristic plant species that are adapted to tidal conditions, like *Caltha palustris* var. *araneosa*, or to a brackish environment, like *Cochlearia officinalis* subsp. *officinalis*, almost disappeared from the Haringvliet estuary.

During the decades following the closure, however, public awareness of the ecological losses increased (Smits *et al.,* 2006; Paalvast, 1998a). For the near future, it has therefore been decided to set the sluices ajar 95% of the time. This is expected to increase the tidal amplitude by 0.1 m and create a salt gradient over a maximum distance of 18 km (Bol & Kraak, 1998). As this will not restore the historical estuarine conditions (for instance, no change in morphodynamics is expected), a more extensive opening of the sluices is envisaged for the distant future, on the assumption of a decreasing interest of agriculture and water extraction companies in this area, allowing an increase of the salt concentration in the water (Lofvers, 1998; Paalvast *et al.* 1998).

Although an environmental impact assessment undertaken to estimate the effects of the changed sluice management did address the mobilisation of heavy metals and organic toxins accumulated in the former estuary, it did not elaborate on the effects of its reopening on nutrient cycles and other biogeochemical processes. Many studies have focussed on the effects of salinity on vegetation and biogeochemical processes, but most of them addressed rather high salinities, and information on the effects of oligohaline conditions is scarce. Although little information is available on these effects and interactions with alterations of the tidal regime, such information is essential to predict the effects of restoration measures in estuaries. In the present study, we simulated the effects of restoring tides and salinity on biogeochemical processes in soils

and vegetation originating from the shores of the Haringvliet. We hypothesised that the following effects could occur:

1. The increased tidal amplitude could change the oxygen availability in the soil, which is very important as it determines whether decomposition of organic matter takes place aerobically or anaerobically. We assumed that in places were the soil surface is flooded twice a day, anaerobic processes would be favoured compared to the stagnant situation with the same mean water table. Reduction of iron in anaerobic decomposition will lead to phosphate mobilisation, as iron(hydr)oxides release part of the adsorbed phosphate when Fe(III) is reduced to Fe(II) (e.g. Patrick & Khalid, 1974). In general, the anaerobic decomposition rates are lower than aerobic decomposition rates (e.g. Gale & Gilmour, 1988; Dauwe *et al.,* 2001). As alternating water tables are known to stimulate the volatilisation of nitrogen by stimulating both nitrification at high redox potentials and denitrification at low redox potentials (e.g. Hefting *et al.* 2004), we expected increased coupling of nitrification and denitrification.

2. Increased salt concentrations after the reopening of the estuary will cause cation exchange between the cation adsorption complex and the brackish water. Additionally, anion exchange might occur. Beltman *et al.* (2000) found that chloride acts as a competitor, increasing the availability of phosphate.

3. High sulphate concentrations in sea salt may lead to greater production of free sulphide, which is toxic to salt marsh vegetation (e.g. Portnoy & Giblin, 1997; Portnoy, 1999). Under iron-rich circumstances, however, most of the sulphide will be bound to iron, preventing toxicity. This can cause additional release of phosphate, as phosphate is outcompeted by sulphide in binding to iron (Caraco *et al.,* 1989; Smolders & Roelofs, 1993; Roden & Edmonds, 1997; Lamers *et al.,* 1998, Loeb *et al.,* 2007).

4. During anaerobic decomposition, CH_4 may be produced. In a brackish environment, however, the higher sulphate concentrations can be expected to inhibit methanogenesis, because they favour sulphate-reducing bacteria (Cappenberg, 1974; Abram & Nedwell, 1978; Lovley & Klug, 1983; Bartlett *et al.,* 1987; Scholten & Stams, 1995).

5. Chloride toxicity may play an important role when the salinity gradient is restored. Freshwater microorganisms are known to perform less well under salinity stress (e.g. Olsen *et al.,* 1996; Coci *et al.,* 2005). Therefore, decomposition of organic matter in formerly freshwater areas may be temporarily reduced, untill the microbial population has adapted to the new environmental conditions. Salt will also have an influence on the vegetation development in the rehabilitated area. Salt stress on plants can be divided into

chloride toxicity, water stress by osmotic potential and nutritional imbalance (Ashraf & Harris, 2004). Salinity-tolerant plants can cope with these stresses in many ways. One of the ways to achieve osmotic adjustment is the production of certain free amino acids (Ahmad *et al.,* 1981; Hodson *et al.*, 1985; Pulich, 1986; Mansour, 2000; Ashraf & Harris, 2004). Salt-tolerant plants will compete better under salt stress than salt-intolerant plant species, resulting in a change in vegetation composition.

We tested the above hypotheses in a full factorial mesocosm experiment and in a batch incubation experiment. We established an increase in tidal amplitude of 0.2 m and an oligohaline salt concentration of 3‰, which are intermediate levels. This is representative of the future situation, i.e. after restoration measures have been taken, at the locations we studied (Bol & Kraak, 1998). As agricultural activity in the Haringvliet area is assumed to decrease in the future (Lofvers, 1998; Smits *et al.,* 2006), part of the agricultural land will be converted into nature reserves. We therefore examined effects on soils originating from a nature reserve and from an agricultural location, both typical of the Haringvliet estuary.

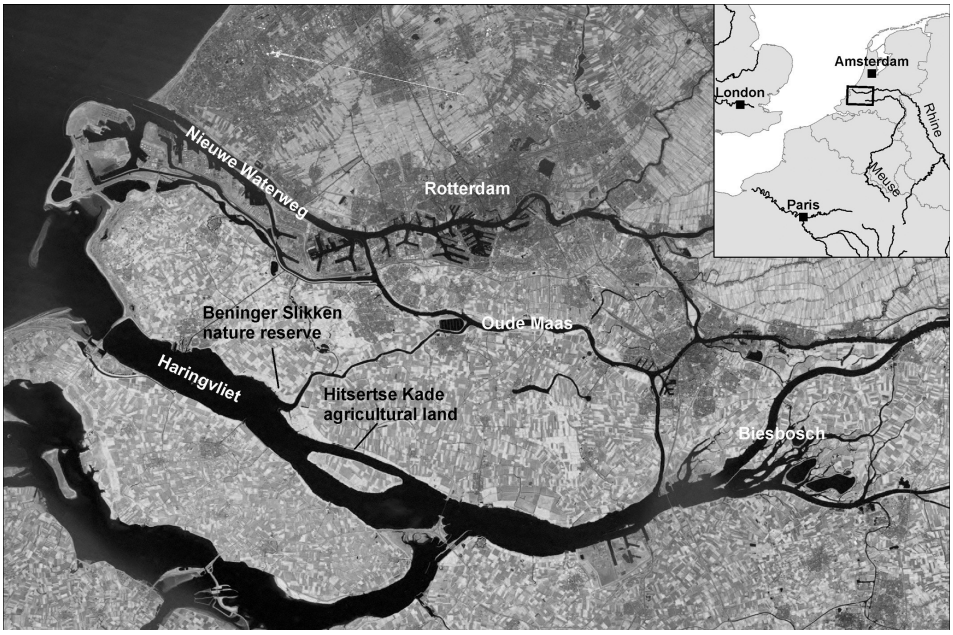

Figure 1. Location of the sampling sites on the shores of the Haringvliet estuary and the reference water bodies Nieuwe Waterweg and Oude Maas. The present tidal amplitude in the Haringvliet and the Biesbosch nature reserve originates from the estuary of the Nieuwe Waterweg, west of Rotterdam, which connects the city's port with the sea.

Table 1. Soil characteristics. Soil extractions are expressed in μmol g^{-1} dry weight. No difference was made between the top soil of AL (agricutlural land) and the deeper layer. Pore water measurements were taken in the top 10 cm of the soil. No separate pore-water extractions were performed on the C-horizon of NR (nature reserve). Pore water measurements are expressed in μmol L^{-1}. Standard errors of the mean are given in brackets.

	extraction method	Nature reserve A-horizon	Nature reserve C-horizon	Agricultural land
% organic matter	loss-on-ignition	16 (1)	5 (0.6)	7 (0.2)
NH_4^+	NaCl	0.23 (0.08)	0.09 (0.02)	0.12 (0.05)
NO_3^-	MilliQ	5.8 (1.7)	1.9 (0.9)	1.1 (0.2)
$P_{available}$	Olsen	2.2 (0.7)	1.9 (0.4)	1.9 (0.2)
P_{total}	digestion	43 (3.9)	24 (2.4)	28 (1.3)
K^+	NaCl	1.0 (0.3)	0.6 (0.1)	2.2 (0.1)
Fe	oxalate	110 (5.6)	68 (1.2)	64 (5.8)
NO_3^-	pore water	4.6 (1)		123 (20)
NH_4^+	pore water	14 (2)		16 (6)
PO_4^{3-}	pore water	3.4 (1)		8.9 (3)
pH	pore water	7.13 (0.03)		7.25 (0.03)
K^+	pore water	50 (13)		497 (83)
Na^+	pore water	2824 (465)		624 (55)
Ca^{2+}	pore water	4588 (888)		1225 (57)
Mg^{2+}	pore water	544 (111)		163 (6)
SO_4^{2-}	pore water	3776 (945)		477 (37)
Cl^-	pore water	2096 (466)		680 (83)

Materials and methods

Site description and sampling

In July 2004, vegetated sods with a surface area of 60 x 40 cm and a height of 20 cm were collected from two locations on the shores of the Haringvliet estuary (Figure 1). The Beningerslikken area (NR) (51°47'24'' N, 4°13'40'' E) is a nature reserve outside the dike, grazed by cattle and geese. Before the estuary was closed off, it used to be an area with tidal flats where reed and bulrush were cultivated. Nowadays, the grazed areas form a species-rich grassland. At the Hitsertse Kade area (AL) (51°45'17''N, 4°18'30''E), which is protected against most flood events by an embankment, sods were taken from a species-poor agricultural grassland, grazed by cattle, sheep and horses. The bottom and sides of the sods were wrapped in root cloth to prevent rooting and to prevent soil being washed out into the surrounding water during the experiment. The sods were then put in perforated plastic containers. Table 1 shows the characteristics of these soils. The nature reserve soil had a distinct A horizon, with an organic matter content of 16%. The deeper layers, as well as the soil from the agricultural land, were poor in organic matter (5–7%). Both locations had intermediate concentrations of amorphous (oxalate-extractable) Fe, compared to other locations in Dutch floodplains, where concentrations amount to 1000 μmol g^{-1} dry weight (Loeb *et al.,* 2007). Higher concentrations

of NO_3^-, PO_4^{3-} and K^+ in the soil pore water of the agricultural land compared to the nature reserve reflect the use of fertilizers. In the nature reserve, concentrations of Cl^-, Na^+, Mg^{2+} and SO_4^{2-} reflect the greater connectivity with the estuarine water, at present as well as in the recent past, compared to the agricultural land. Note that this did not hold for the extraction values, and that the concentration of total P was higher for the nature reserve sods, in agreement with the higher organic matter concentration.

Experimental set-up and sampling

Shortly after sampling, the containers with the sods were placed in outdoor water basins in Nijmegen, the Netherlands, with a capacity of approximately 2500 L (180 cm diameter, 100 cm height; Figure 2). In each basin, one sod from each location was placed on top of concrete tiles to adjust the soil surface of the sods to the water level. Treatments were applied in a full factorial design with either 0 or 0.2 m tidal difference and a salinity of 0.1 or 3‰, to test the effects of tidal amplitude, salinity and possible interactions. Each treatment had four replicates. The non-tidal basins were filled with 1200 L of water, and the elevation of the sods was adjusted to a water level 10 cm below the soil surface. A pump ensured water circulation. Each tidal basin was connected by hoses to three interconnected reservoirs per basin, with a total content of 900 L. Each day, at 12 am and 12 pm, water was pumped into the reservoirs over a period of 4–6 hours. At 6 am and 6 pm, the water was pumped from the

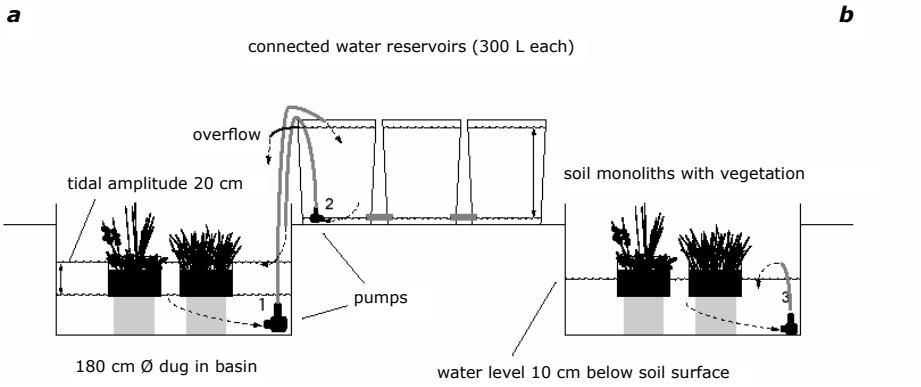

Figure 2. Experimental set-up of the tidal (a) and non-tidal (b) treatments. In each basin, sods from both the nature reserve and the agricultural grassland were placed. Pump 1 in the tidal treatment was active during ebb-tide, while pump 2 initiated high tide. Pump 3 in the non-tidal treatment was working continuously to maintain water circulation. (Adapted from Antheunisse *et al.* 2007).

Table 2. Composition of the surface waters at Haringvliet, Nieuwe Waterweg (reference site for brackish tidal area) and Oude Maas (reference site for freshwater tidal area), and of the surface water at the beginning of the experiment.

	Haringvliet	Nieuwe Waterweg	Oude Maas	Artificial brackish water	Artificial fresh water
Na^+ (mmol L^{-1})	1.6	39	2.1	36	2.6
K^+ (mmol L^{-1})	0.1	0.9	0.1	0.9	0.1
Ca^{2+} (mmol L^{-1})	1.7	2.1	1.9	2.1	0.9
Mg^{2+} (mmol L^{-1})	0.4	4.4	0.5	4.3	0.5
NO_3^- (mmol L^{-1})	0.6	0.05	0.09	0.3	0.3
PO_4^{3-} (µmol L^{-1})	1.5*	0.4	2	0.4	0.4
Cl^- (mmol L^{-1})	2.1	37.7	2.5	37.1	2.7
SO_4^{2-} (mmol L^{-1})	0.6	2.5	0.6	2.7	0.6
pH	7.6	8.0	8.0	8.7	8.7
alkalinity (meq L^{-1})	2.5	2.7	2.9	2.1	1.7

∗ at Haringvliet, total dissolved P was measured.

reservoirs into the basins again over a period of 4–6 hours, generating a tidal difference of 0.2 m. The mean water level in the tidal basins was adjusted to 10 cm below the soil surface of the sods.

Artificial oligohaline water and fresh water were made by adding artificial sea salt (Meersalz Professional, Wiegandt GmbH, Krefeld, Germany) to tap water to simulate the concentrations of Cl^-, Na^+, Ca^{2+}, Mg^{2+} and SO_4^{2-} in either oligohaline water from a brackish tidal reference site (Rozenburg, Nieuwe Waterweg) or freshwater from a freshwater tidal reference site (Heijnenoord and Spijkenisse, Oude Maas) (Table 2). Initial nitrate concentrations were higher than in the reference water, due to higher concentrations in the tap water. They did not, however, differ much from the current nitrate concentrations in the Haringvliet lake. Nitrate disappeared from the basins within three to seven weeks. Water levels and salt concentrations were adjusted when they had changed as a result of rain or evaporation. Unfortunately, the results of one of the freshwater tidal replicates had to be removed from the dataset, because the basin had a leak, which meant tap water had to be continuously added, resulting in higher nitrate concentrations.

Before inserting the sods into the basins, the species composition and abundance of plants in each sod were carefully described and three rhizon samplers with a length of 10 cm (Rhizon SMS-10cm; Eijkelkamp Agrisearch Equipment, The Netherlands) were placed diagonally in each of the sods to prevent infiltration of surface water. At the start of the experiment and at each sampling event, pore water was taken through these samplers into 30 mL vacuumed infusion flasks. Samples from each sod were pooled to reduce the effects of heterogeneity. In addition, a 1 L surface water sample was taken next to the sods, using a polyethylene bottle. Water samples were taken weekly at the

start of the experiment, and monthly or bi-monthly later on (12 times in total). In August 2004, vegetation height was estimated. At the end of the growing season (October 2004), aboveground biomass was harvested from a 0.04 m^2 subsample of the sods, to determine biomass production and tissue nutrient concentrations. At the end of the experiment, in July 2005, new vegetation relevés were recorded and vegetation height was estimated, after which the aboveground standing stock was harvested. Leaves of *Agrostis stolonifera* and *Mentha aquatica* were used for amino acid analysis.

At the end of the experiment, soil samples were taken from each sod to measure potential decomposition rates in the laboratory. We assumed that decomposition in the mesocosm experiment had been partly aerobic and partly anaerobic, regardless of the tidal regime (in the tidal treatments because of the changing water table and in the non-tidal treatments because the top 10 cm of the sod had been situated above the water table and the rest of the sod below it). We therefore incubated each soil sample both aerobically and anaerobically, to enable analysis of the effects of salinity and of the previous tidal regime on decomposition. 50 g portions of fresh sediment were transferred to 350 mL glass infusion flasks. The flasks were filled up to 170 mL with water of the same composition as that used in the mesocosm experiment. Concentrations in the demineralised water medium were adjusted to the concentrations in the mesocosm experiment using artificial sea salt (Meersalz Professional, Wiegandt GmbH, Krefeld, Germany), $NaHCO_3$, $CaCl_2$ and Na_2SO_4. The headspaces of the anaerobic incubations were flushed with N_2. Bottles were sealed with airtight rubber stoppers and shaken in the dark at 20 °C. Samples from the headspace were taken twice a week with 1 mL syringes and were analysed instantaneously for CO_2 and CH_4. After 32 days, headspace samples were taken for H_2S analysis. Two days later, slurry water samples for the determination of potential mineralisation rates were taken, using rhizon samplers (Rhizon SMS-5cm; Eijkelkamp Agrisearch Equipment, The Netherlands). Potential decomposition rates were calculated by linear regression of the net production in the linear phase of the CO_2 production. CH_4 production did not add to the potential decomposition rate, because the production of CH_4 did not start until after the linear phase of the CO_2 concentration build-up. Potential CH_4 production rates were calculated in the same way. Potential net mineralisation rates were calculated from the change in pore water concentrations.

Chemical analyses

pH was measured with a Radiometer Copenhagen type PHM 82 standard pH meter. Alkalinity was determined by titration of 10 mL samples with 0.1 mmol L^{-1} HCl down to pH 4.2. Turbidity of the surface water was measured with a

Dentan Turbidimeter (model FN-5). For the purpose of ICP analyses, concentrated nitric acid was added up to a concentration of 1.17% to the samples to keep the oxidised iron in solution. Samples were stored in polypropylene tubes at 4°C. For all other analyses, 0.12 g citric acid L^{-1} was added to the samples. They were stored in iodated polyethylene bottles at -24°C until further analyses. Total concentrations of Ca, Mg, P, Fe and S were analysed by ICP-MS (X series, Thermo Electron Corporation) and ICP-OES (Spectroflame, VML2 and IRIS Interpid II XDL, Thermo Electron Corporation). Total Fe and Mn measured in the pore water was considered to represent Fe^{2+} and Mn^{2+}, since these are the dominant soluble species at the pH we measured. Total S was considered to represent SO_4, since, at the concentrations occurring in our experiment, only a very small part will be in organic form. o-PO_4 (Technicon AutoAnalyser II), NH_4^+, NO_3^- (+ NO_2^-) and Cl^- (Bran+Luebbe AutoAnalyser 3) were analysed colorimetrically using ammonium molybdate, salicylate, hydrazine sulphate and ferriammonium sulphate, respectively. CO_2 and CH_4 were measured by infrared analysis (ABB Advance Optima Infrared Gas Analyser). H_2S was measured with a gas chromatograph (Chrompack, CP 9001).

Total concentrations of elements other than N in plant and soil materials were determined by digesting 200 mg of homogenised and dried sample (24 h, 70°C) for 17 minutes in 4 mL concentrated HNO_3 and 1 mL 30% H_2O_2 (Milestone microwave MLS 1200 Mega). Samples were analysed by ICP-OES (Spectroflame, VML2). N in the plant tissue was determined using Kjeldahl digestion, modified with salicylic acid thiosulphate (Bremner & Mulvaney, 1982), using 150 mg of the samples. The concentration of N was measured colorimetrically on a continuous flow analyser (SA-40, Skalar Analytical, Breda, The Netherlands).

The organic matter content of the sediments was determined by loss-on-ignition (4h, 550°C). Soil extractions were performed on fresh sediments and corrected for moisture content afterwards, after drying (24h, 105°C). The concentration of amorphous Fe was determined by oxalate extraction (2.5 g of soil shaken for 2h with 30 mL of a solution containing 16.2 g of $(COONH_4)2.H_2O$ and 10.9 g of $(COOH_2).2H_2O$ per L) (Schwertmann 1964), and plant-available P by bicarbonate extraction (5 g of soil shaken with 100 mL of 0.5 M $NaHCO_3$) (Olsen *et al.* 1954). NaCl extraction was performed using 100 mL 0.1 M NaCl per 35 g of soil. In the MilliQ extraction, 35g of soil was shaken with 100 mL MilliQ.

The amino acid analysis used leaves of *Agrostis stolonifera* (from both locations) and *Mentha aquatica* (only from the sods from the nature reserve) collected from various plants per replicate and immediately frozen at -24 °C until analysis. Samples were ground in a frozen state with liquid N_2 and amino acids were extracted from 0.5 g fresh sample with 20 mL extraction solution contain-

ing ethanol, thiodiglycol and citric acid (Van Dijk & Roelofs, 1988). 600 nmol norleucine was added as an internal standard. After extraction, ethanol was removed using chloroform. Extracts were analysed with HPLC (Varian Star).

Statistics

All statistics were calculated with SPSS 13.0 (SPSS Inc., 2004). Significance was accepted at a confidence level of $p \leq 0.05$. For effects with known direction (the effect of salinity on biomass and vegetation height and the effect of tides on methane production rate and on concentrations of amino acids known to increase with salinity), 1-tailed significance was accepted. Concentrations of ions in the pore water and surface water, production rates and concentrations of nutrients in the plant tissue were log (x+1) transformed to make them fit normal distribution better. Effects of time series were tested with General Linear Method (GLM) repeated measures, using Greenhouse-Geisser corrections if terms of sphericity were not met in Mauchly's test. Correlations between the concentrations in pore water and surface water were calculated non-parametrically by Spearman's correlation, significance being accepted at a confidence level of $p \leq 0.01$. Other effects of salinity and tide and the differences between the two soil types were tested using a Mann-Whitney U test or an ANOVA test (the latter indicated if used). All non-parametric tests were performed on non-transformed data.

Results

Soil pore water and surface water

Time had a significant influence on almost all parameters measured (GLM repeated measures). Some concentrations, such as that of calcium, changed over time to a new equilibrium, while others, such as iron (Figure 3) and phosphate (results not shown), showed a clear seasonal variation, with lower concentrations between December and April, when temperatures were lower. Tidal treatment (GLM repeated measures, between-subject effects) had a significant effect on the soil pore water concentrations of Cl^- ($p = 0.000$), Ca^{2+} (only in the sods from the agricultural land, $p = 0.002$), Fe^{2+} ($p = 0.008$) (Figure 3) and PO_4^{3-} ($p = 0.020$). Concentrations of Cl^- were significantly higher in the tidal systems, whereas concentrations of Ca^{2+}, Fe^{2+} and PO_4^{3-} were lower. The concentrations of PO_4^{3-} and Ca^{2+} in the surface water were, however, somewhat higher in the tidal treatments ($p = 0.000$ and 0.038 respectively). Salinity increased the pore water concentrations of ions added with the sea salt (S (only significant in the agricultural soil, $p = 0.000$), Na^{2+} ($p = 0.000$), Cl^- ($p = 0.000$), Mg^{2+} ($p = 0.000$) and K^+ ($p = 0.000$), results not shown). Addition-

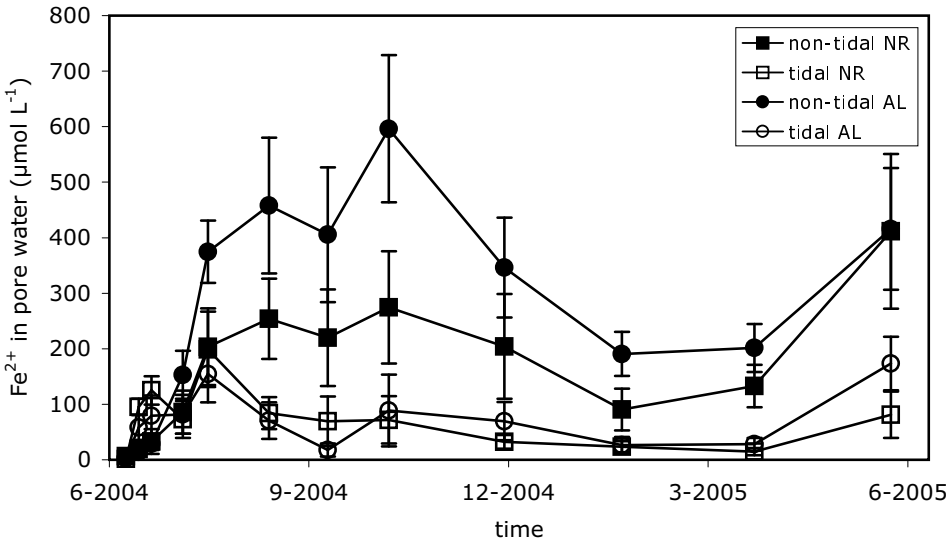

Figure 3. Iron concentrations in the pore water during the experiment in the sods from the nature reserve and agricultural land. Squares: nature reserve; circles: agricultural land; closed symbols: non-tidal treatments; open symbols: tidal treatments. Measurements of salinity treatments were pooled. Error bars represent the standard error of the mean.

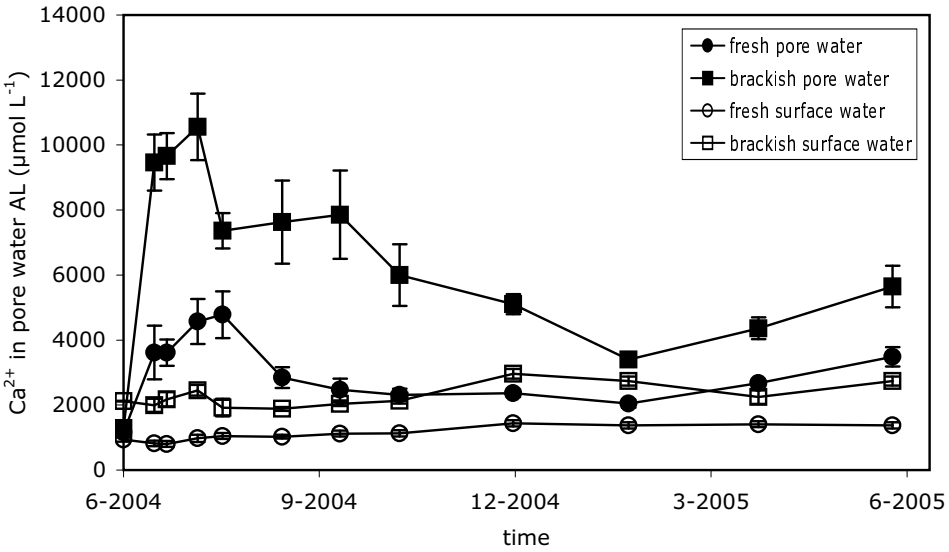

Figure 4. Calcium concentrations in the surface water and in the pore water of the sods from the agricultural land (AL). Open symbols: surface water; closed symbols: pore water of AL; circles: fresh water treatments; squares: oligohaline treatments. Measurements of tidal treatments were pooled. Error bars represent the standard error of the mean.

ally, concentrations of Ca^{2+} increased (p = 0.000) (Figure 4 for the agricultural land). Initial concentrations of Ca^{2+} in the soil pore water were approximately 4500 µmol L^{-1} for the nature reserve and 1500 for the agricultural land. Surface water concentrations of $Ca^{2=+}$ in the brackish treatment were around 2300 µmol L^{-1} and those in the fresh water around 1100 µmol L^{-1}. Pore water concentrations in the brackish treatment, however, did not converge towards surface water concentrations over time, but rose to values of approximately 6000 µmol L^{-1} at the end of the experiment, i.e., approximately 2.5 times the surface water concentration.

Elements added with the salt (Na, K, Cl, Mg, S) were, as expected, strongly intercorrelated both in the surface water and in the pore water. Products of anaerobic metabolism (Fe^{2+}, Mn^{2+} and NH_4^+) also showed high degrees of intercorrelation in the soil moisture. These products also correlated positively with a higher alkalinity in the pore water: the correlation coefficient (ρ) in the soil from the nature reserve was 0.674 for Fe and 0.821 for Mn, while ρ in the soil from the agricultural land was 0.678 for Fe and 0.808 for Mn, as a result of consumption of protons in these processes. PO_4^{3-} in the soil moisture had the highest correlation with Fe^{2+} (nature reserve soil ρ = 0.446, agricultural soil ρ = 0.520). No positive correlations were found between the PO_4^{3-} concentration in the pore water and the sulphate or chloride added with the salt (nature reserve soil ρ = –0.079 p =0.299; agricultural soil ρ = –0.220 p = 0.04). Turbidity, caused by algal bloom in the first few months of the experiment, correlated with a high pH (0.651) and a low alkalinity (-0.237), as a result of CO_2 depletion by photosynthesis.

Decomposition

Potential decomposition rates in the aerobic incubations were, on average, 3 times higher than in the anaerobic incubations (p = 0.000) (Figure 5). Increased salinity reduced the aerobic decomposition rate in the agricultural soil significantly (7.5 for the freshwater and 4.4 µmol C g^{-1} dw day^{-1} for the oligohaline treatments) (p = 0.016). The salinity effect was not significant for the soil from the nature reserve, nor for the anaerobic incubations in both soils.

The potential CH_4 production rate was influenced by the tidal treatment to which the sods had been exposed during the year preceeding the anaerobic decomposition experiment (Figure 6), despite the fact that all soils were incubated anaerobically. The samples originating from sods subjected to the tidal treatment in the mesocosm experiment had a much lower production rate (0.6 µmol CH_4 g^{-1} dw day^{-1}) than those from the non-tidal treatment (1.2 µmol CH_4 g^{-1} dw day^{-1}) (ANOVA, p = 0.034 1-tailed). The lag phase of the methane pro-

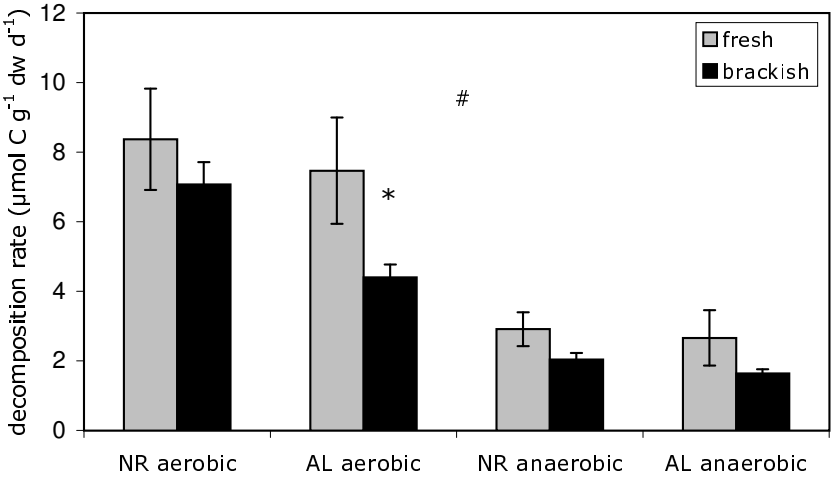

Figure 5. Potential decomposition rates for the nature reserve (NR) and the agricultural land (AL) in aerobic and anaerobic incubations of the fresh and brackish treatments. Grey bars: freshwater treatments; black bars: oligohaline treatments. Previously tidal and non-tidal treatments were pooled. Error bars represent the standard error of the mean. Significant differences between brackish and freshwater treatments and between aerobic and anaerobic incubations are indicated by *; significant differences between aerobic and anaerobic treatments are indicated by #.

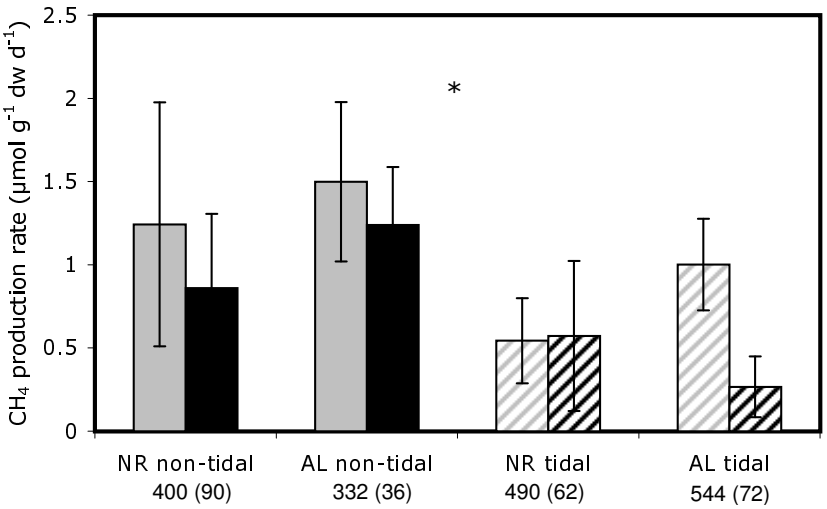

Figure 6. Potential methane production rates in the anaerobic incubations. Grey bars: freshwater treatments; black bars: oligohaline treatments; hatched bars: previously tidal treatments. Error bars represent the standard error of the mean. Numbers below the figure show the duration of the lag phase (hours) (standard error of the mean in brackets, fresh and brackish treatments pooled). A significant difference between non-tidal and tidal treatments is indicated by *.

duction (i.e. the phase in which no production took place) was also extended in the incubations with samples from tidal treatments ($p = 0.039$). Although salinity seemed to decrease methane production rates (-29% on average) and increase the lag phase (+32%) of the methane production in the anaerobic treatments (Figure 6), these effects were not significant ($p = 0.258$ and 0.119, respectively).

H_2S production measured at the end of the decomposition experiment (Figure 7) was higher in the slurry from the agricultural land than in that from the nature reserve ($p = 0.000$). The brackish treatment produced more H_2S ($p = 0.010$), especially in the agricultural soil. Despite the differences, concentrations of H_2S in the headspace were very low in all samples.

During the anaerobic decomposition, potential net mineralisation rates of Fe^{2+}, Mn^{2+} and NH_4^+ in the soil from the nature reserve were higher, and potential net PO_4^{3-} mineralisation rates lower, than in the agricultural soil (data not shown).

Vegetation

No effect of treatment was found on the plant species composition or species abundance after a year of exposure to tides and salinity. Species richness decreased in all treatments; that in the sods from the nature reserve from an average of 14.9 to 11.4 species and that in the sods from the agricultural land from 3.4 to 2.6 species. Species tolerant to oligohaline conditions, such as *Glaux maritima*, *Althaea officinalis* and *Odontites vernus*, which were present in the vegetation of the sods from the nature reserve, did not increase at the expense of purely freshwater species within this timespan. The only species that decreased substantially, in all treatments, was *Cirsium arvense*.

Although the standing above-ground biomasses of the sods from the agricultural land and the nature reserve did not differ significantly (781 and 876 kg m^{-2}, respectively; see also Antheunisse *et al.* 2007), the height of the vegetation was significantly lower ($p = 0.000$) on the sods from the agricultural land than on the sods from the nature reserve. This can be explained by the species composition, as the agricultural grassland is dominated by *Agrostis stolonifera*, whereas the nature reserve featured several tall herbs, members of the sedge family and reed. In August 2004, i.e., two months after the start of the experiment, the salinity treatment had reduced the maximum vegetation height by 17% on the nature reserve sods ($p = 0.001$ 1-tailed) and by 14% on the agricultural land sods ($p= 0.047$ 1-tailed). The aboveground biomass harvested in October 2004 (Figure 8) was also reduced by salinity, both in the nature reserve sods (26%, $p = 0.020$ 1-tailed) and in the agricultural land

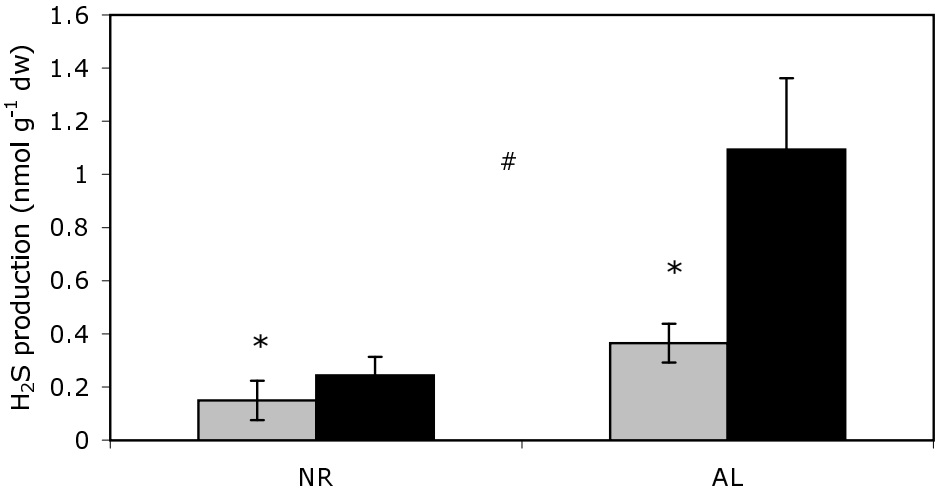

Figure 7. H_2S production in the anaerobic incubations. Grey bars: freshwater treatments; black bars: oligohaline treatments. Previously tidal and non-tidal treatments were pooled. Error bars represent the standard error of the mean. Significant differences between brackish and freshwater treatments are indicated by *; significant differences between soils from the nature reserve (NR) and the agricultural land (AL) are indicated by #.

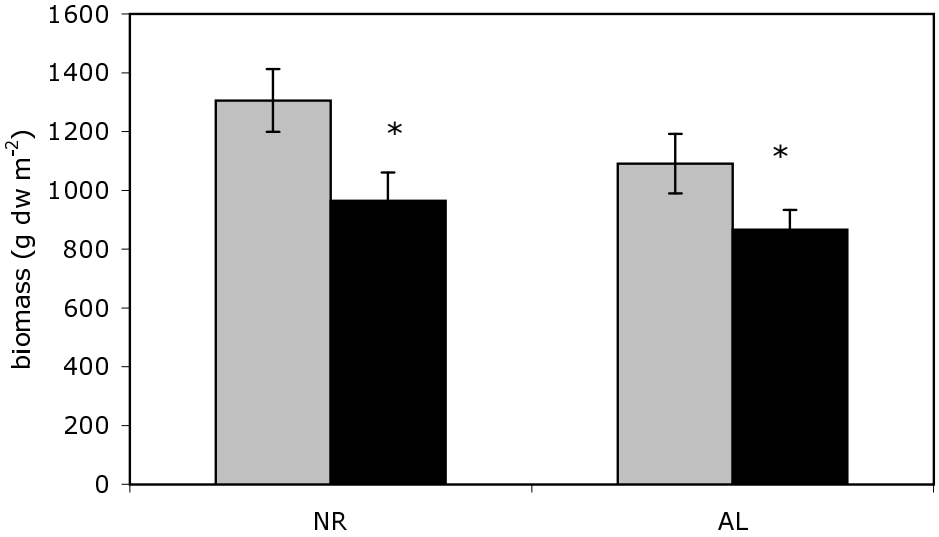

Figure 8. Aboveground biomass at the end of the first growing season on the sods from the nature reserve (NR) and from the agricultural land (AL). Grey bars: freshwater treatments; black bars: oligohaline treatments. Tidal and non-tidal treatments were pooled. Error bars represent the standard error of the mean. * indicates significant differences between the brackish and freshwater treatments.

sods (21%, p= 0.047 1-tailed). At the end of the experiment in June 2005, no significant effect of salinity on biomass was present anymore, but average vegetation height was still reduced by 16% (on the nature reserve sods) and 21% (on the agricultural land sods). At this time, the vegetation biomass on the agricultural soil had also been slightly (7%, p = 0.040) reduced by the tidal movement. The vegetation on the sods from the nature reserve, on the other hand, seemed to be stimulated (27% more biomass) by the tidal regime, but this effect was not significant (p = 0.094). No significant effects of tides on vegetation height were found.

Initial soil characteristics determined the concentrations of many nutrients in the vegetation. At the end of the first growing season, concentrations of N and P were higher in the vegetation from the agricultural soil, whereas concentrations of Ca, Mg, Na and S were higher in the vegetation from the nature reserve soil, regardless of the treatment (data not shown). Unexpectedly, no effects of salinity were found on element concentrations in the plant tissue, not even on the concentrations of the elements added with the sea salt, such as Na, S and Mg, even though they had increased in the pore water. The amino acid concentrations, however, showed a clear effect of salinity in the leaf tissue of *Agrostis stolonifera* (Figure 9). Salinity increased the concentrations of asparagine (p = 0.002, 133% increase), proline (p = 0.047, 70% increase) and serine (p = 0.004, 51% increase). Nitrogen in the increased amino acids made only a small contribution to total N (with a mean combined concentration of proline, asparagine and serine amino acid N in *Agrostis stolonifera* in the brackish treatments of 4.4 µmol g^{-1} dw and a mean total N in the vegetation of 460 µmol g^{-1} dw). *Mentha aquatica* showed no effect of salinity on amino acid concentrations in the leaf tissue.

Discussion

Mixing of soil pore water and surface water

The tidal movement caused an increased mixing of surface and pore water. Whereas in the non-tidal treatments, rainwater was able to form an upper zone with lower chloride concentrations, in the tidal treatments, chloride from the surface water was fully transported into the root zone, exposing the vegetation of the brackish tidal treatments to a higher average chloride concentration. Reversely, in the tidal treatments, ions from the pore water were flushed out to the surface water, resulting in higher concentrations of phosphate and calcium in the surface water.

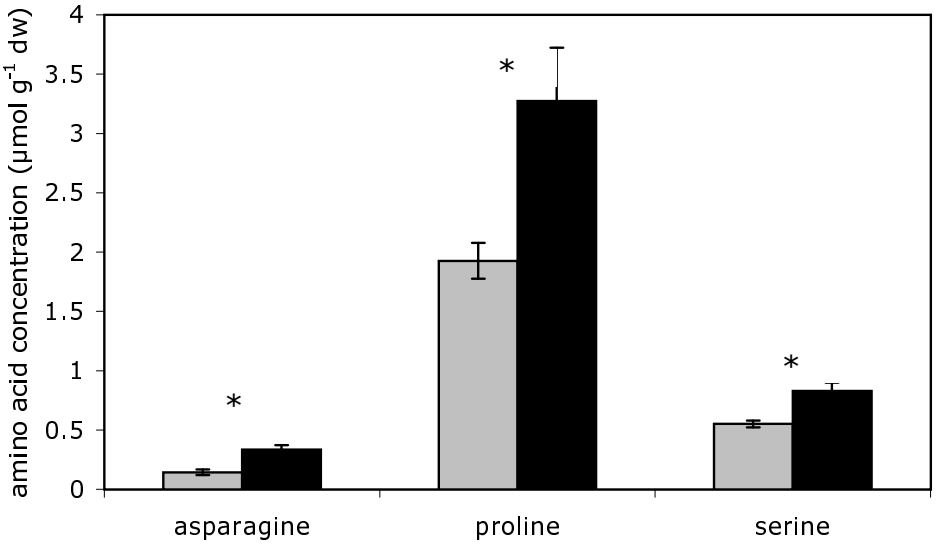

Figure 9. Free amino acid concentrations in leaves of *Agrostis stolonifera*. Grey bars: freshwater treatments; black bars: oligohaline treatments. Measurements of both locations were pooled. Error bars represent the standard error of the mean. * indicates significant differences between the brackish and freshwater treatments.

Redox potential

Contrary to our expectations, the results indicated a generally higher redox potential in the tidal treatments. The lower concentrations of reduced iron in the pore water indicate that oxygen was better able to penetrate into the soil at low tide. Accordingly, generally lower iron reduction rates led to reduced mobilisation of phosphate from the iron(hydr)oxides-phosphate complexes and hence to lower concentrations of P in the pore water, as mentioned above (Patrick & Khalid, 1974; Loeb *et al.,* in press). The oxygen availability also repressed the activity of methanogenic bacteria, resulting in the lower and delayed production of methane in the anaerobic incubations. In the same mesocosm experiment, Antheunisse *et al.* (2007) found higher N mineralisation rates and lower denitrification rates for the tidal treatments in this experiment, also pointing at a higher redox potential and oxygen availability with increased tidal amplitude. Hence, the expected increase in N loss as a result of stimulated coupling of nitrification and denitrification did not occur. Similar results were obtained by Thompson *et al.* (1995), who found much lower denitrification rates in a restored tidal salt marsh consisting of course sands due to higher oxygen intrusion compared to a more silty natural marsh, although both soils had similar nitrification rates.

Ion exchange

Increased salinity caused cation exchange between the surface water, the pore water and the cation adsorption complex of the soils, resulting in much higher calcium concentrations in the soil pore water of the oligohaline treatments than in the surface water. Calcium was exchanged with sodium, magnesium and potassium from the sea salt. The fact that concentrations of calcium in the pore water were still high at the end of the experiment indicates that after a year, equilibrium with the surface water had not been reached. The higher calcium concentrations in the surface water of the tidal treatment indicate that an increased tidal amplitude will accelerate this process in the field. Cation exchange is also known to be an important process determining the availability of NH_4^+ in estuaries, as this monovalent ion is easily outcompeted by other cations, which are present in high concentrations in the haline water (Seitzinger *et al.,* 1991; Rysgaard *et al.,* 1999). Beltman *et al.* (2000) found anion exchange between chloride and phosphate in peat soils at even lower chloride concentrations than those applied in our oligohaline treatments. We did, however, not find any correlation between the phosphate concentration in the pore water and the chloride concentration, so we conclude that anion exchange did not play any role here in phosphate mobilisation.

Influence of sulphate on sulphide toxicity and phosphate mobilisation

Sulphate, an important component of the sea salt, was reduced to sulphide in the anaerobic incubations. Since the concentrations of sulphide in the headspace and in the slurry water were very low, we conclude that most of this ion was precipitated with reduced iron (Caraco *et al.,* 1989; Lamers *et al.,* 1998; Loeb *et al.,* 2007). This conclusion is also supported by the fact that the agricultural soil, having a lower iron concentration in the pore water and lower concentrations of amorphous iron in the top soil, and therefore probably a lower iron availability, produced more H_2S. In both soils, free sulphide in the water phase was calculated to be a factor of 105 - 107 smaller than potentially phytotoxic levels (circa 10 µmol L^{-1}) (Smolders & Roelofs, 1996). Therefore, sulphide toxicity is not expected to play a major role for the vegetation.

Just as in the mesocosms, no significant effects of salinity on phosphate mobilisation rates were found in the incubation experiment. It has to be remembered, however, that sulphate availability in this batch experiment was limited, whereas in the field, fresh sulphate would be supplied with every high tide. On the other hand, the mesocosm experiment, in which a surplus of sulphate was present, shows that sulphide production does not play an important role in the availability of phosphate. Phosphate in the pore water was not correlated with

the sulphate added with the salt. In addition, the suppressed iron reduction in the tidal treatments makes sulphate reduction under a tidal regime not very plausible, as iron is a better electron acceptor than sulphate. Additionally, high salt concentrations in the field will be accompanied by a high tidal amplitude, much higher than that used in our mesocosm experiment. We therefore assume that, if any FeS_x is formed by the reduction of sulphate in the top soil, it will be oxidised during low tide. Miletto *et al.* (in review) found that in samples from the agricultural soil taken from the same mesocosm experiment and incubated anaerobically with a surplus of sulphate, it took several days before sulphate reduction started, in all treatments. This makes sulphate reduction in the top layer in the field even more unlikely. It is possible that additional formation of FeS_x will take place in deeper soil layers where no oxygen is present, under the influence of the increased salinity and accompanying increased sulphate concentrations, thus causing a permanent change in Fe speciation, as this FeS_x will not be oxidised again (Canavan *et al.,* 2006). This may lead to higher concentrations of phosphate in the pore water in deeper soil layers.

Decomposition and mineralisation

As expected, aerobic decomposition was much faster than anaerobic decomposition. Antheunisse *et al.* (2007) also found higher actual N mineralisation rates in the tidal treatments. It should be noted that other processes than the mineralisation of organic matter play a more important role in the net release of some elements. We found high net release rates for Fe^{2+}, Mn^{2+}, NH_4^+ and PO_4^{3-} in the anaerobic incubations due to their release by anaerobic metabolism. We expect that restoration of the tidal movement will increase the decomposition and mineralisation rates in the part of the soils and sediments that is now lying beneath the water table but will be exposed to oxygen again after the restoration of the tidal regime. Although in the nature reserve most organic matter is concentrated in the top layer of the soil, the layer beneath the top soil still contains approximately 5% organic matter. It has also been shown that sediment close to the Haringvliet dam still contains 4% organic matter at a depth of 7.5 m below the water surface (Carnavan *et al.,* 2006). We therefore expect that overall decomposition rates in the Haringvliet area will be stimulated by setting the sluices ajar.

Methane production

The lower and delayed production of methane in the anaerobic incubations of soil from the tidal treatments shows that the tidal regime not only influenced the oxygen supply, but also the activity of the methanogenic archaea. Methane

production may also be decreased by the higher sulphate supply that went with the higher salinity, although this was not significantly supported by our findings. Sulphate is known to suppress methanogenesis, because it favours sulphate-reducing bacteria over methanogenes (e.g. Cappenberg, 1974; Canavan *et al.,* 2006; Loeb *et al.,* 2007). Middelburg *et al.* (2002) found that dissolved methane concentrations in the surface water of European rivers decreased with higher salinity. However, they also found very high concentrations of methane in tidal flats, where methane production is probably higher because of the higher organic matter production compared to the rest of the estuary (Middelburg *et al.,* 2002). An increase in the tidal amplitude at the Haringvliet estuary may yield a larger area suitable for the growth of helophytes. This may stimulate methane production due to increased input of litter and facilitate methane emission through gas transfer from soil to atmosphere through the shoots. Accordingly, the Haringvliet area may still be an important source of methane releases into the atmosphere.

Chloride toxicity

Aerobic decomposition was reduced under the influence of salinity in the agricultural soil. Aerobic microorganisms are known to be hampered by chloride toxicity (Olsen *et al.,* 1996; Coci *et al.* 2005). The agricultural site has been closed off from the estuary for many decades, which may have changed the composition of the microbial population at this site over the years. Although the effect on the aerobic decomposition rate was still increased after the year of oligohaline exposure, Miletto *et al.* (in review) showed that after three months, the composition of the anaerobic sulphate-reducing microbial community in this mesocosm experiment was already significantly different between the treatments within each soil type, indicating the adaptation of the microbial community to the newly created conditions. We expect that the aerobic bacteria population in agricultural lands that are transformed into new nature reserves will also adapt to the new, oligohaline situation, gradually reducing the effect of the salinity on aerobic decomposition.

Salinity reduced the biomass production during the first growing season. At the end of the experiment, biomass production did not differ significantly from that in the freshwater treatments, but the higher concentrations of the amino acids asparagine, proline and serine in the leaf tissue of *Agrostis stolonifera* in the oligohaline treatments indicate that the vegetation was still experiencing salt stress. The amino acids we found to increase with salinity were also found by Ahmad *et al.* (1981) to do so in a salt-tolerant and a freshwater ecotype of *Agrostis stolonifera*, indicating their role in salt tolerance. Concentrations of amino acids in our experiment were much lower than values found in the

literature (Ahmad *et al.,* 1981; Hodson *et al.,* 1985). Hodson *et al.* (1985) showed that increased salinity reduced the growth of *Agrostis stolonifera* more strongly under nitrogen-poor conditions, and that these amino acids accumulated less under nitrogen-limited conditions. In our experiment, nitrate concentrations in the water were low, and the low N:P ratios (Antheunisse *et al.,* 2007) also suggest nitrogen limitation (Koerselman & Meuleman, 1996). In the field, on the other hand, there will be a small but continuous nitrate supply. Therefore, the toxic effects of the restored salinity may be less severe in the field than in our mesocosm experiment. In fact, even in the mesocosms, the effect of nitrogen limitation on chloride toxicity seemed to be small, as the tissue nitrogen concentration of the vegetation in the mesocosm experiment was not increased by salinity, unlike what has been reported in literature about nitrogen-limited conditions (Hodson *et al.,* 1985). For these reasons, we do not expect that there will be any interactive effects of nitrogen limitation and salinity in the field.

Biomass production and vegetation composition

In spite of the probably higher availability of nitrogen in the tidal treatments, aboveground biomass production was not significantly stimulated by the tidal regime. On the agricultural soil, biomass production was slightly hampered by the tides. This is not surprising, since many plant species are not adapted to submerged conditions. Large parts of the Haringvliet area will have an even larger tidal amplitude than in our experiment. The timespan of the present study was too short to observe changes in vegetation composition. It can, however, be expected that restoration of the salinity gradient, leading to the kind of salt stress demonstrated in this paper, and that of the tidal regime, will lead to a shift to more characteristic plant species. Provided diaspore supply is not a constraint, we expect species composition in the field to shift to species adapted to these riparian circumstances, such as *Phragmites australis*, *Scirpus lacustris*, *Mentha aquatica* and hopefully also characteristic species such as *Caltha palustris araneosa* in the freshwater and *Scirpus maritimus* and *Cochlearea officinalis* in the brackish zones.

Conversion of agricultural land to nature reserve

Although differing in vegetation type as well as in soil characteristics, the soil from the nature reserve and from the agricultural land showed similar biogeochemical responses. The slightly lower aerobic decomposition rate in the agricultural soil was supposedly temporary, as described above.

Conclusion

In conclusion, we expect that both restored salinity and particularly restored tidal movement, increasing aerobic processes, will significantly influence biogeochemistry in estuarine areas. The higher redox potentials partly prevent mobilisation of phosphate, although the tidal movement may increase exchange of phosphate to the surface water. Also aerobic decomposition will increase due to the higher oxygen availability. In addition with higher nitrification rates, this may lead to a higher nutrient availability for the vegetation. Sulphate reduction and methane production are suppressed, preventing additional release of phosphate and methane emission to the atmosphere, respectively. An increase of salinity will lead to cation exchange in formerly freshwater wetlands and therefore a possibly higher leach-out of calcium and ammonium. Restoration of oligohaline conditions leads demonstrably to chloride toxicity in the vegetation.

Acknowledgements

We would like to thank M. Versteeg, R. van de Haterd, S. Sollie, R. van den Heuvel, A. Lawniczak, A. Sigterman, R. Zeijpveld and G. van de Weerden for their help in the field and at the experimental site. We would also like to thank J. Eygensteyn, E. van Daalen and A. Pol for their help with the chemical analyses, and the Vereniging Natuurmonumenten and the Holster family for their kind permission to collect the sods from their lands. We thank J. Klerkx for his linguistic advice. This study was financially supported by the Netherlands Organisation for Scientific Research (NWO) as a part of the TRIAS programme (project 835.80.010).

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N+P
18

Nutrient limitation along eutrophic rivers? Roles of N, P and K input in a species-rich floodplain hay meadow

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Accepted by Applied Vegetation Science

Abstract

Question: Is the growth of biodiverse floodplain plant communities along nutrient-rich lowland rivers still limited by nutrients?

Location: Floodplains of the river Overijsselse Vecht, the Netherlands.

Methods: Soil characteristics and potential nutrient limitation of the vegetation types were studied in two hay meadows, both belonging to the *Fritillario-Alopecuretum*, in the floodplain of the eutrophic river Overijsselse Vecht (the Netherlands). The meadows had different fertilisation histories: one was a species-rich hay meadow managed as a nature reserve, the other a newly created nature reserve that had been used as an agricultural pasture before. Sods collected from both locations were transferred to a greenhouse and fertilised weekly with N+P, N+K, P+K, N+P+K or control solutions, and harvested 3 times a year during 2 successive growing seasons.

Results: Biomass production of sods from both locations of this floodplain appeared to be still limited by N. Interestingly, the sods from the existing nature reserve were also limited by K, but only in the second year. Fertilisation caused a shift in the relative abundance of the different plant families. Tissue nutrient concentrations were increased by fertilisation with the nutrient in question, but decreased if biomass production was stimulated.

Conclusions: Even in eutrophic river areas, the nutrient concentrations of the surface water may still determine the development of potentially biodiverse floodplain vegetation.

Introduction

Rivers are among the most highly impacted ecosystems in the world (Dynesius & Nilsson, 1994). Although the floodplains of lowland river courses are naturally rich in nutrients (Vannote *et al.,* 1980; Spink *et al.,* 1998), eutrophication of the river water and river sediments and direct agricultural fertilisation of floodplains have led to eutrophication of river floodplains along anthropogenically influenced rivers (Grevilliot *et al.,* 1998; Lamers *et al.,* 2006). In temperate regions worldwide nitrogen fluxes in rivers increased 2 - 20 fold due to anthropogenic input (Howarth *et al.*, 2006). The type of nutrient limitation (i.e. the nutrient that can increase biomass production) in these eutrophied systems is often unclear. In highly dynamic systems, such as frequently flooded sites within floodplains, disturbance events rather than competition of plants for nutrients determine the composition of the vegetation (Grime, 1974). Beltman *et al.* (2007) found an *Arrhenatherion* plant community in a floodplain along the river Rhine to be limited by nutrients in some years, whereas disturbance by flooding determined vegetation productivity in other years. The vegetation of less disturbed floodplains has been found to be limited by various nutrients, or was found no longer to be limited by nutrients. Floodplain grasslands of the rivers Allier, Loire and Rhine are limited by N (Van Oorschot *et al.,* 1998; Beltman *et al.,* 2007) or co-limited by N and P (Rhine) (Beltman *et al.,* 2007). Intermediately productive meadows along the smaller Dutch river Dommel were found to be limited by N, co-limited by N and P, by N and K, and by P and K, depending on the type of plant community (Olde Venterink *et al.,* 2001). However, other authors found no impact of fertilisation on river floodplain vegetation at all, due to the fact that the input of nutrients was so high that biomass production was no longer stimulated by additional nutrients (Spink *et al.,* 1998; Antheunisse *et al.,* 2006).

Determining the type of nutrient limitation is important for habitat management, as plant species diversity (often one of the aims of nature rehabilitation programmes) and biomass production are often closely correlated. Grime (1973a, b) showed that the upper limit of the cloud of points describing biomass and species richness at a certain site, is hump-shaped. In the higher productivity range, where floodplain vegetations are situated, input of nutrients favours fast-growing plant species, which outcompete other species, resulting in lower species richness. Conversely, species richness can be promoted by decreasing biomass production by lowering the availability of the limiting nutrient. Or, in the case of protection of biodiverse plant communities, by preventing extra input of the limiting nutrient. Of course, not all plant species within a plant community have to be growth-limited by the same nutrient (Braakhekke & Hooftman, 1999; Güsewell *et al.,* 2003), so the input of a nutrient that is not limiting biomass production as a whole may still favour certain plant species

over others, which may result in a shift in species composition (Güsewell *et al.,* 2003; Güsewell, 2004).

One of the ways to determine the limiting nutrient(s) is by means of fertilisation experiments, either in the field or with vegetated sods taken from the field (Olff & Pegtel, 1994). Caution is required when determining single-species limitations from species biomass responses in fertilisation experiments, as species are subject to competition (Güsewell *et al.,* 2003). From a management point of view, determining the nutrient limitation of a single species is, however, less relevant than the effects of fertilisation on the species abundance within the vegetation and on the species composition of the plant community as a whole, which is a result of both limitation and competitive strength. Another option to determine the type of nutrient limitation is to measure nutrient ratios in the plant tissue (Koerselman & Meuleman, 1996; Verhoeven *et al.,* 1996; Güsewell *et al.,* 2003; Olde Venterink *et al.,* 2003). Koerselman and Meuleman (1996) found distinct boundaries in N:P ratios between N- and P-limited sites. These boundaries were confirmed and sharpened by several other studies (Güsewell & Koerselman, 2002; Olde Venterink *et al.,* 2003). Although plant tissue concentrations can differ widely, irrespective of the type of nutrient limitation (Güsewell & Koerselman, 2002), the ratios between concentrations of nutrients do seem to indicate the type of limitation. Besides ratio boundaries, indicating N or P limitation, boundaries for K limitation or co-limitation, derived from N:K and P:K ratios, have also been found (Olde Venterink *et al.,* 2003). For single plant species, however, the response to fertilisation cannot be derived from critical N:P ratios (Güsewell *et al.,* 2003). Studies have found not only critical nutrient ratios, but also critical nutrient concentrations: plant communities of the temperate zone seem to be limited by N below an overall plant tissue concentration of 9.5 mg N g^{-1}, and by P below a concentration of 0.5 mg P g^{-1}, whereas P limitation never seems to occur when plant tissue concentrations are above 1 mg P g^{-1} (Güsewell & Koerselman, 2002).

As common plant communities along eutrophic river often seem not to be limited by nutrients anymore, we were interested whether this also applies for more rare species-rich grasslands. As an example we chose two grasslands along the river Overijsselse Vecht in the Netherlands. This is a medium-sized river with a basin of approximately 4000 km^2 in Germany and the Netherlands, and is polluted with nitrogen (Anonymus, 2004). In the future, greater connectivity between floodplains and rivers in Western Europe can be expected, due to more frequent (summer) floods as a result of global climate change (Christensen & Christensen, 2003; Kundzewicz *et al.,* 2005). This may lead to higher nitrogen loads on floodplains along nitrogen-enriched rivers such as the Overijsselse Vecht.

Materials and Methods

Site description

Plant communities of the two studied grasslands belong to the endangered *Fritillario-Alopecuretum pratensis* community (Horsthuis *et al.,* 1994), characterised by the bulb species *Fritillaria meleagris*. This species has the centre of its distribution in the Netherlands, but the present size of the population in this country is estimated to be only 5% of its size in 1900 (Horsthuis *et al.,* 1994). The *Fritillario-Alopecuretum pratensis* community is found in floodplain hay meadows on clay and silt soils, often on deeper peat layers, with a high groundwater table in winter and spring, which are flooded 2–4 times a year (Zuidhoff *et al.,* 1996). These requirements are met in the lowest freshwater ranges of lowland rivers (Zuidhoff *et al.,* 1996).

One of the floodplain grasslands we studied (Huis den Doorn (HDD); 52°33'18''N6°7'22''E) is a well-preserved hay meadow, which has been managed as a nature reserve for many decades. It is mown two times a year. The other grassland (Zwartsluis (ZWS); 52°37'40''N6°4'58''E) was used as a pasture for cattle for many decades and has been managed as a nature conservation area for just a few years. It is mown once a year and is grazed at the end of the growing season, followed by the application of fresh manure (less than 20 * 10^3 kg ha^{-1}). The presence of *Fritillaria meleagris* in this hay meadow is declining (personal communication J. Breedenbeek). On May 3, 2006, 2x2 m vegetation relevées were made in the field, and soil samples were collected. At each location three soil samples, consisting of at least five subsamples with a depth of about 15 cm, were taken from an area of approximately 20 m^2 with an auger. Of each relevée the plant community was determined by Associa (Synbiosys 1.11, Alterra, Wageningen). Vegetation relevées are shown in Table 1. Nomenclature follows Van der Meijden (2005). The vegetation of the HDD grassland (nature reserve) is a typical *Fritillario-Alopecuretum pratensis* hay meadow. The vegetation of ZWS (abandoned pasture) could also be characterised as a *Fritillario-Alopecuretum pratensis* hay meadow, but also contained species of *Calthion palustris* plant communities (Zuidhoff *et al.,* 1996), such as *Lychnis flos-cuculi*, *Carex disticha* and *Persicaria amphibia*.

Organic matter contents of the soils were determined by loss-on-ignition (4h, 550°C). Soil extractions were performed on fresh soils and corrected for moisture content after drying (24h, 105°C). NaCl extraction was performed using 100 ml 0.1 M NaCl per 35 g of soil. In the MilliQ (H_2O) extraction, 35g of soil was shaken with 100 ml MilliQ. The concentration of amorphous Fe, which is supposed to represent the fraction available for microbial Fe reduction, was determined by oxalate extraction (2.5 g of soil shaken for 2h with 30 mL of

Table 1. Vegetation relevées of the hay meadows, according to the combined Braun-Blanquet scale (Westhoff *et al.,* 1995).

Plant community	HDD *Fritillario-Alopecuretum typicum*	ZWS *Fritillario-Alopecuretum calthetosum*
Total coverage	100%	70%
Mean height (cm)	20	15
Total number of species (4 m^2)	19	21
Alopecurus pratensis	2b	2a
Anthoxanthum odoratum	2a	2b
Bellis perennis		2a
Cardamine pratensis	2a	1
Carex acuta	3	2b
Carex disticha		2a
*Cerastium fontanum*vulgare*	r	
Festuca rubra	1	
Fritillaria meleagris	2a	+
Galium palustre		1
Glechoma hederacea		+
Holcus lanatus	1	
Juncus acutiflorus		r
Lathyrus pratensis	+	
Leontodon autumnalis	+	
Lychnis flos-cuculi		2a
Lysimachia nummularia		1
Plantago lanceolata	2b	2a
Poa trivialis	2a	2b
Polygonum amphibium		+
Ranunculus acris		2a
Ranunculus auricomus	2b	
Ranunculus ficaria	2a	+
Ranunculus repens	2a	1
Rumex acetosa	1	+
Taraxacum spec	2a	+
Trifolium pratense	2a	
Trifolium repens		1
Vicia cracca	+	

a solution containing 16.2 g of $(COONH_4)_2.H_2O$ and 10.9 g of $(COOH_2).2H_2O$ per L) (Schwertmann 1964) and plant-available P by bicarbonate extraction (5 g of soil shaken with 100 mL of 0.5 M $NaHCO_3$) (Olsen *et al.* 1954). CEC and base saturation were determined by a triple sequential extraction of 5 g of fresh soil with a 0.1 M $BaCl_2$ solution, followed by extraction with a 0.020 M $MgSO_4$ solution. The CEC was calculated from the excess Mg in this extract. Base saturations of elements were calculated from their respective concentrations (charge equivalents) in the $BaCl_2$ extract relative to the CEC. In order to determine the total concentrations of elements in soil materials, 200 mg of ground, homogenised and dried sample was digested for 17 minutes with 4 mL concentrated HNO_3 and 1 mL 30% H_2O_2 (Milestone Ethos D - Microwave

Labstone). Total concentrations of Ca, Fe, K, Mg, P and S were analysed by ICP-OES (IRIS Intrepid II XDL, Thermo Electron Corporation). o-PO_4 (Technicon AutoAnalyser II), NH_4^+ and NO_3^- (+ NO_2^-) (Bran+Luebbe AutoAnalyser 3) were analysed colorimetrically using ammonium molybdate, salicylate and hydrazine sulphate, respectively.

The soil of ZWS was a clay soil (25-35% lutum) on a sandy subsoil, at 20 cm beneath the soil surface. The soil at HDD contained on average more organic matter (n.s.), more silt, and somewhat less clay than the soil at ZWS. Table 2 presents the soil characteristics. Concentrations of K were more than 2 times higher in the ZWS soil. Remarkable are the high concentrations of Fe (total and amorphous) in both soils, especially at HDD, compared to soils from other Dutch floodplains (Loeb *et al.,* 2007; Loeb *et al.* in press). Due to their historically low elevation relative to surrounding peatlands, these floodplains probably received iron-rich groundwater discharge in the past. Nowadays, these peatlands have been drained and have subsided, and groundwater discharge to the floodplains has stopped. The pH of both soils was slightly acidic (pH 5.5 - 6) after extraction with demineralised water, which is rather low for floodplain soils in the Netherlands, but base saturation of both soils was high (around 87%), which means that they are well-buffered against acidification.

Table 2. Soil characteristics, concentrations in μmol /g DW, CEC in cmol+ kg^{-1}, base saturation in mol %, organic matter in mass %. Standard error of the mean shown in brackets. Asterisks mark significant differences between the soils.

	HDD	ZWS
Organic matter (mass %)	12.2 (0.3)	9.6 (1.1)
NO_3^- – H_2O	0.43 (0.08)*	0.18 (0.01)
NH_4^+ – NaCl	0.12 (0.003)*	0.43 (0.12)
total N	372 (20)	336 (19)
K^+ – NaCl	0.06 (0.002)	0.28 (0.14)
total K	22 (1)*	50 (1)
PO_4 – Olsen	0.35 (0.31)	0.63 (0.08)
total P	37 (1)*	28 (1)
Fe – oxalate	459 (45)*	320 (23)
total Fe	702 (2)*	532 (29)
pH – H_2O	5.95 (0.13)	5.45 (0.23)
CEC	24 (0.9)	22 (1)
Base saturation Ca (%)	82 (1)*	62 (3)
Base saturation Mg (%)	3.8 (0.04)*	16 (0.9)
Base saturation K (%)	0.2 (0.02)	0.9 (0.4)

Experimental set-up

Sods with a diameter of 24 cm and a height of 18 cm were taken from both grasslands on March 30, 2005. They were transported to an unheated greenhouse in Nijmegen, the Netherlands, where they were placed in plastic containers with holes in the bottom covered by root cloth. The pots were placed on plastic dishes, which were kept wet with demineralised water to enable capillary rise of water. Two delivery devices were placed in each sod for automatic watering. This was done to prevent the sods from shrinking and to avoid the vegetation drying out by evaporation during hot periods. All sods received the same amount of demineralised water at the same time, but the quantity was adjusted depending on the climatic conditions at the time of watering.

Five different fertilisation treatments were applied to sods from both hay meadows (five replicates): +PK, +NP, +NK, +NPK and a control treatment (only demineralised water added). Every week, each sod was fertilised according to one of these treatments, receiving 250 mL demineralised water with N added as 497 µmol L^{-1} $NaNO_3$ (20 kg N ha^{-1} $year^{-1}$), P as 56 µmol L^{-1} Na_2HPO_4 (5 kg P ha^{-1} $year^{-1}$) and/or K as 178 µmol L^{-1} KCl (16 kg K ha^{-1} $year^{-1}$). The control sods received 250 mL demineralised water without added nutrients. Fertilisation started in the first week of April 2005. The experiment was ended after two growing seasons, in September 2006.

Harvests and plant analyses

In April 2005, July 2005, October 2005, April 2006, July 2006 and September 2006, the aboveground living biomass was clipped. Except for the October 2005 and July 2006 harvests, aboveground biomass was split into the 10 major plant families occurring on these sods: *Asteraceae*, *Brassicaceae*, *Caryophyllaceae*, *Cyperaceae*, *Fabaceae* (*Leguminosae*), *Juncaceae*, *Plantaginaceae*, *Poaceae*, *Polygonaceae*, *Ranunculaceae*, and a category 'others', to enable the response of different plant families to the different types of fertilisation to be analysed. Harvest was dried at 70 oC for 48 h and weighed. In April 2005, April 2006 and September 2006, plant tissue nutrient concentrations of plants belonging to the four families with the highest biomass – *Cyperaceae*, *Plantaginaceae*, *Poaceae* and *Ranunculaceae* – were analysed, and in October 2005 and July 2006, plant tissue nutrient concentrations were analysed in the vegetation as a whole.

Total concentrations of elements in plant material were determined following the same procedure as with the soil analysis. C and N concentrations in the plant material (20 mg) were determined with a CNS analyser (Carbo Erla Instruments NA 1500).

Statistics and calculations

Effects of treatments were tested on ln(x+1) transformed data to achieve a closer fit of the data to a normal distribution. Overall effects in time were calculated per location with GLM repeated measures, using Greenhouse-Geisser corrections if terms of sphericity were not met in Mauchly's sphericity test. Differences in biomass and plant tissue nutrient concentrations between treatments per location were calculated with a Tukey post-hoc test following ANOVA. Differences in biomass and plant tissue nutrient concentrations between treatments between the HDD and ZWS locations were calculated with a t-test. All statistics were calculated with SPSS 15.0 (SPSS Inc.). Significance was accepted at a probability level of $p \leq 0.05$. Plant tissue nutrient concentrations of the total vegetation from the harvests subdivided by family (October 2005 and July 2006), were calculated by taking the weighted average of the four families which made up at least 80% of the total biomass. The effects of the treatments on the performance of individual families were evaluated using absolute and relative biomass data of the harvests of the second year (i.e. combining those of April and September 2006), because we expected differences in species composition to appear after the first year of the experiment.

Results

Biomass of the total vegetation

Both soils had low dry weights at the first harvests, due to the low temperatures in the field in the weeks before the sods were brought to the greenhouse (Figures 1a and 1b). The second harvest already yielded significantly higher biomass on sods from HDD in the +NP treatment than in the control treatment (Figure 1a; Table 4). In the subsequent harvests in October 2005 and April 2006 the aboveground biomasses of the +NK and +NPK treatments were significantly higher than the biomass in the +PK treatment. However, in July 2006, biomass was lower in the +NP and control treatments than in the +NK and +NPK treatments. In addition, the biomass of the control treatment was lower than that in the +PK treatment. In the final harvest, only the control treatment had a lower biomass than all other treatments.

The sods from ZWS showed a different pattern (Figure 1b; Table 3 and 4). As from October 2005, there were significant differences between the +NK and +NPK treatments on the one hand and the control treatment on the other. Later on, differences became more pronounced, showing lower biomasses for the +PK and control treatments compared to the +NK, +NP and +NPK treatments. In September 2006, differences between control and fertilisation treat-

ments became smaller, so no significant effects of the fertilisation were found anymore. Vegetation biomass was, on average, higher for HDD sods than for ZWS sods (p= 0.000).

Nutrient concentrations in the total vegetation

On sods from HDD, concentrations of N in plant tissue were significantly higher in the control and +NP treatments (Figure 2; Table 5). On ZWS sods, overall concentrations of N were significantly higher in the +NP treatment than in the +PK and +NPK treatments, but this was mainly due to significant differences in the final harvest. Concentrations of N on ZWS sods were significantly lower than on HDD sods (19.0 mg N g^{-1} against 20.3 mg N g^{-1}, p= 0.05). All N concentrations remained above 9.5 mg g^{-1}.

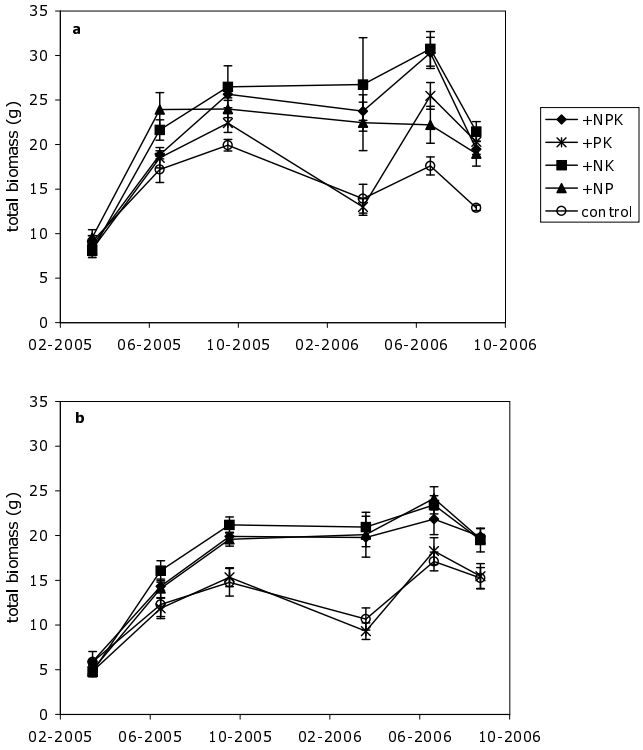


Figure 1a and 1b. Biomass development (regrowth after harvest) on the sods from Huis den Doorn (HDD) (a) and from Zwartsluis (ZWS) (b) for the five different fertilisation treatments. Error bars represent standard error of the mean (n=5).

Table 3. Overall effects (p-values) of treatments on total aboveground biomass (repeated measures, between-subjects effects) and results of Tukey post-hoc test. Different letters indicate significant differences in biomass between different treatments within a location (a = lowest biomass, c = highest).

location	HDD	ZWS
between-subjects effects (overall effect of treatment)	0.000	0.000
	posthoc between-subjects effects	
+NPK	bc	b
+PK	ab	a
+NP	bc	b
+NK	c	b
control	a	a

Table 4. Effects (p-values) of treatments on total aboveground biomass (ANOVA) and results of Tukey post-hoc test within the separate harvests. Different letters indicate significant differences in biomass between different treatments within a location (a = lowest biomass, c = highest).

harvest	Apr 2005		Jul 2005		Oct 2005	
location	HDD	ZWS	HDD	ZWS	HDD	ZWS
Effect of treatment	0.704	0.337	0.012	0.040	0.012	0.004
	posthoc effects					
+NPK	a	a	ab	a	b	ab
+PK	a	a	ab	a	ab	a
+NP	a	a	b	a	ab	ab
+NK	a	a	ab	a	b	b
control	a	a	a	a	a	a
harvest	Apr 2006		Jul 2006		Sep 2006	
location	HDD	ZWS	HDD	ZWS	HDD	ZWS
Effect of treatment	0.009	0.000	0.000	0.000	0.000	0.018
	posthoc effects					
+NPK	ab	b	c	bc	b	a
+PK	a	a	bc	ab	b	a
+NP	ab	b	ab	c	b	a
+NK	b	b	c	c	b	a
control	a	a	a	a	a	a

Starting from October 2005 (ZWS) or April 2006 (HDD), harvest concentrations of P in the vegetation on both soils were significantly lower in the +NK treatment than in the other treatments. The +PK treatment on ZWS sods and the +NP treatment on HDD sods resulted in a significantly higher concentration of P in the plant tissue than the +NPK treatments on the sods from these locations. Concentrations of P were all above 1 mg P g^{-1}. Concentrations of P did not differ significantly between the sods from the two locations.

Table 5. Overall effects (p-values) of treatments on plant tissue nutrient concentrations (repeated measures, between-subjects effects) and results of Tukey post-hoc test. Different letters indicate significant differences in concentrations of a nutrient between different treatments within a location (a = lowest concentration, c = highest). Treatments in which the measured nutrient is added with the treatment are shown in bold print.

nutrient	N		P		K	
location	HDD	ZWS	HDD	ZWS	HDD	ZWS
between-subjects effects (overall effect of treatment)	0.000	0.007	0.000	0.000	0.000	0.000
	posthoc between-subjects effects					
+NPK	**a**	**a**	**b**	**b**	**b**	**b**
+PK	a	a	**bc**	**c**	**c**	**c**
+NP	**b**	**b**	**c**	**bc**	a	a
+NK	**a**	**ab**	a	a	**b**	**bc**
control	b	ab	bc	b	a	a

In the above-ground vegetation from both locations, treatments with K added (+PK, +NK and +NPK treatment) had higher concentrations of K than treatments without K addition (+NP and control), starting from April 2006. The vegetation of the +PK treatments had even higher concentrations of K than the +NK (only for HDD sods) and +NPK treatments (both locations). Concentrations of K in the vegetation were significantly higher in the sods from the ZWS soil (13.1 mg g^{-1} against 9.29 in the HDD soil, p= 0.000).

Vegetation nutrient ratios

Nutrient ratios (Figures 2g-2l) between N and P in vegetation from both soils all remained below 14 (except for the +NK treatment in HDD in April 2006 (14.4)), and were highest in the +NK treatments on both soils, due to the lower concentrations of P in vegetation tissue in this treatment.

Starting from October 2005, the ratios between N and K in HDD started to rise in the treatments that did not receive K (+NP and the control treatment). The N:K ratio of the treatments which did receive K stayed around 2 (highest for the +NPK treatment in April 2006 with a ratio of 2.4 on average). On ZWS sods, also the treatments not receiving K had N:K ratios higher than 2.1 in 2006 (and the +NP treatment also in October 2005). For the +NP treatment the N:K ratio was 4 on average in April 2006. The difference in N:K ratios between the locations was caused both by the lower K concentrations, as well as by the higher N concentrations in the vegetation of the sods from HDD. On both soils, the +NP and control treatments showed a significantly higher N:K ratio than the treatments with K added. On ZWS sods, the +NP treatment had a higher N:K ratio than the control treatment in October 2005 and April 2006.

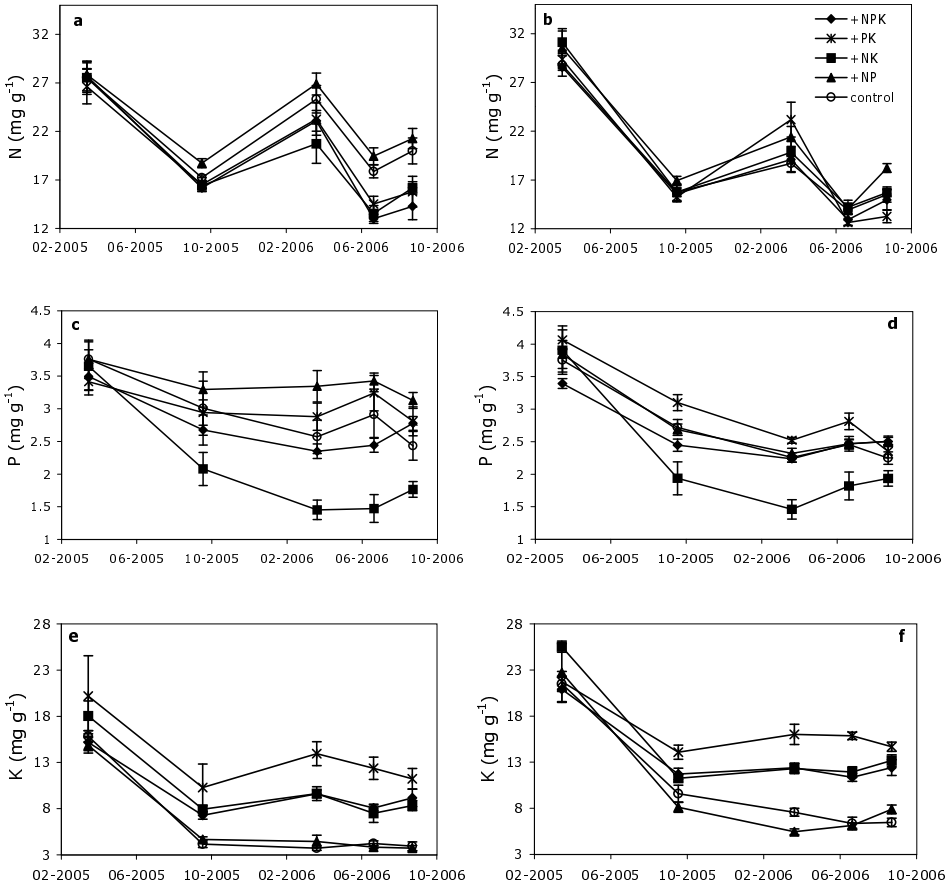


Figure 2. Development of plant tissue nutrient concentrations and ratios in vegetation of sods from Huis den Doorn (HDD) (left; a, c, e, g, i, k) and Zwartsluis (ZWS) (right, b, d, f, h, j, l) for the five different fertilisation treatments. Error bars represent standard error of the mean (n=5).

The P:K ratios showed the same pattern as the N:K ratios. P:K ratios on HDD sods were above 0.29 in the +NP and control treatments and in some of the measurements of the +NPK and +PK treatments. On ZWS sods, P:K ratios in the +NP treatment and the +NPK treatment were above 0.29 during the final year of harvests.

Biomasses of the individual families

The treatments had significantly different effects on the biomass development of the families *Brassicaceae*, *Caryophyllaceae*, *Cyperaceae*, *Poaceae*,

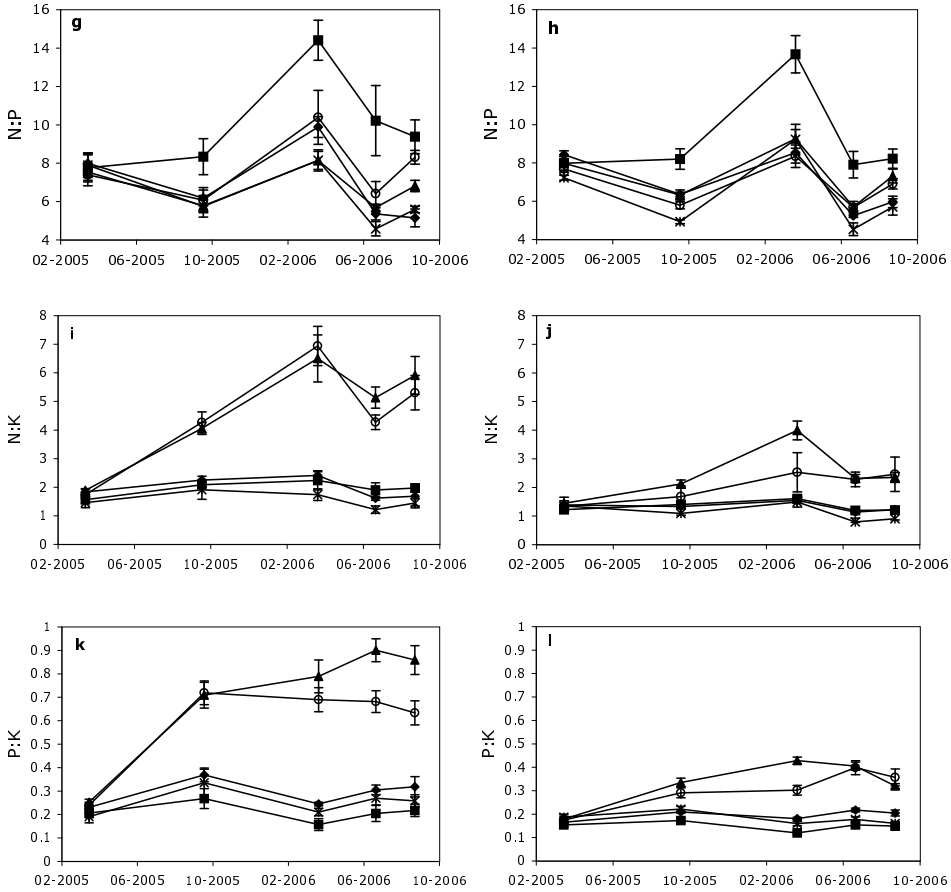


Figure 2. (continued).

Polygonaceae and *Ranunculaceae* in the second year (Figures 3a, c, e, g, i and k). On both HDD and ZWS sods, the *Poaceae* biomass was stimulated by the +NK compared to the +PK treatment. The *Poaceae* biomass was 2.5 to 3.5 times higher in the +NK than in the +PK treatment for both HDD and ZWS. On the sods from ZWS, other treatments also yielded significant results for the *Poaceae* species: biomasses in the +NPK as well as +NP treatments were higher than those in the +PK and control treatments. On ZWS sods, the *Caryophyllaceae* responded negatively to the +PK treatment, but the difference was only significant compared to the +NK treatment. On HDD sods, the *Brassicaceae* (mainly *Cardamine pratensis*) yielded a lower biomass in the +PK treatment, but a higher biomass in the +NP treatment, clearly showing the effect of competition. The fact that the +NP treatment yielded a higher biomass

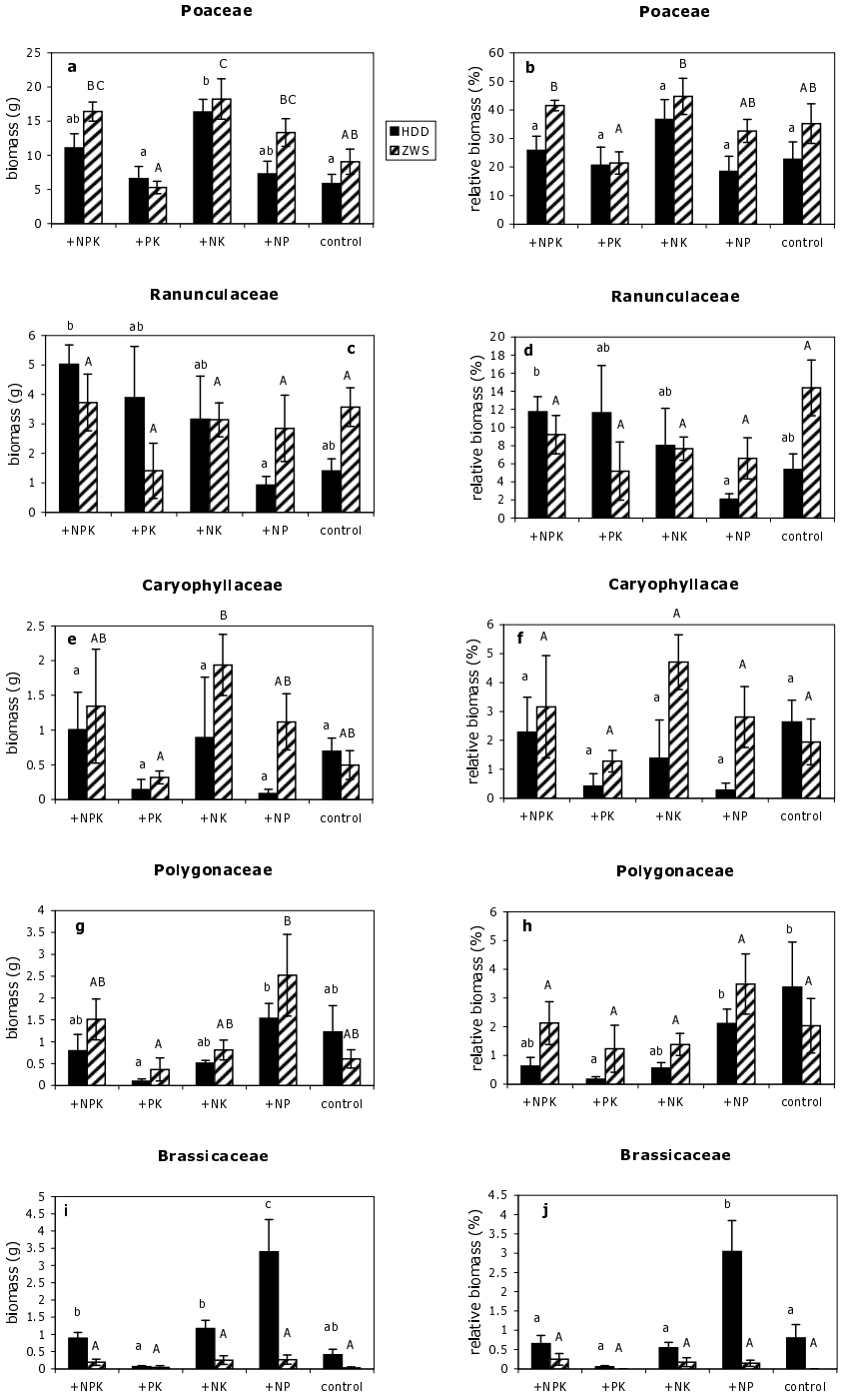
than the +NPK treatment indicates that the *Brassicaceae* took advantage of other families performing worse in the absence of K fertilisation. The *Ranunculaceae* on HDD sods showed a clear effect of the +NP treatment compared to the +NPK treatment: biomasses of this family were more than 5 times lower in the +NP treatment. The *Polygonaceae* performed worse in the +PK treatments than in the +NP treatments.

Of course, positive effects of the treatments on the absolute biomass of a particular family do not necessarily mean that this family is a stronger competitor in these treatments. If the total biomass increases more than that of the family, this means that other families perform better. We therefore also tested the effects of the treatments on the relative biomasses of the families (Figures 3b, d, f, h, j and l). These relative biomasses of the plant families showed the same patterns as the absolute biomasses, though differences were less pronounced.

Nutrient concentrations and ratios in the individual families

In general, the tissue nutrient concentrations and nutrient ratios of the different plant families showed the same patterns as the concentrations and ratios in the total biomass in the various treatments (results not shown). Nevertheless, some clear differences between the plant families were noted. The overall N:P ratios in the *Poaceae* (6.4) and the *Ranunculaceae* (6.6) were lower than those in the *Plantaginaceae* (9.1) and the *Cyperaceae* (12). N:K ratios were lowest in the *Poaceae* (1.6), somewhat higher in the *Ranunculaceae* (2.1) and *Cyperaceae* (2.2) and highest in the *Plantaginaceae* (4.0). The high mean N:K ratios for the *Plantaginaceae* were caused by low concentrations of K at the start of the experiment. Remarkably, where the other families showed a decrease in K concentrations over time (just like the concentrations of K in the total vegetation (Figures 2e and 2f)), even when K was applied, *P. lanceolata* showed a stable concentration of K in the +NK and +NPK treatments, and even an increase in K concentration in the +PK treatment.

Figure 3 (opposite page). Biomass of five plant families in 2006 (combining April and September harvests) subjected to five different fertilisation treatments. On the left: absolute biomass per pot; on the right: relative biomass. Different letters indicate significant differences between treatments within a location. Lower case letters refer to Huis den Doorn (HDD), upper case letters to Zwartsluis (ZWS). Error bars represent standard error of the mean (n=5).


Poaceae
a
biomass (g)
HDD
ZWS
+NPK
+PK
+NK
+NP
control
Poaceae
b
relative biomass (%)
Ranunculaceae
c
biomass (g)
Ranunculaceae
d
relative biomass (%)
Caryophyllaceae
e
biomass (g)
Caryophyllacae
f
relative biomass (%)
Polygonaceae
g
biomass (g)
Polygonaceae
h
relative biomass (%)
Brassicaceae
i
biomass (g)
Brassicaceae
j
relative biomass (%)

Discussion

Nutrient limitation

The growth of the aboveground biomass on sods from both locations was stimulated by fertilisation with N during the first year. Plant tissue N:P ratios in the vegetation (lower than 14) also pointed at N limitation (Koerselman & Meuleman, 1996; Verhoeven *et al.,* 1996; Güsewell *et al.,* 2003; Olde Venterink *et al.,* 2003). All concentrations of N remained above 9.5 mg g^{-1}, which is not conclusive, but P concentrations in the plants were always above 1 mg P g^{-1}, which makes P limitation unlikely (Güsewell & Koerselman, 2002). In the second year, vegetation from ZWS was still stimulated by N fertilisation, while biomass growth for HDD was also stimulated by fertilisation with K in July and September. In July 2006, the biomass production was co-limited by N and K, as was shown by the fact that the highest yield was found on the sods fertilised with both N and K. Fertilisation with either N or K still stimulated biomass production compared to the control treatment, and their effects seemed to be additive. Although in September 2006, all fertilisation treatments stimulated the biomass production equally, co-limitation with P is very unlikely considering the low N:P ratios and the high plant tissue P concentrations.

Critical N:K ratios

During the experiment N:K ratios in the vegetation of the HDD sods rose in the treatments where no K was added. In July and September 2006, when significant effects of K addition on biomass production were found, N:K ratios in these treatments were above 4.3. In October 2005 and April 2006 N:K ratios in these treatments were around 4.2 and 6.8 respectively without showing significant signs of K (co-) limitation. It could, however, be argued whether at that time biomass data already showed a trend towards K (co-) limitation. In other words, determination of the critical lower limit of the N:K ratio from these measurements might not be conclusive. In HDD the other treatments, where K was added, had a maximum ratio of 2.4. In ZWS results show clear evidence for N limitation and did not show any signs of K (co-) limitation in any stage of the experiment. N:K ratios in the vegetation of these sods rose to values of 4 on average for the +NP treatment in April 2006. Also in other treatments and harvests N:K ratios rose above the suggested critical value of 2.1 g g^{-1} (control treatment 2.5 in April 2006; 2.3 in July; 2.5 in September; +NP treatment 2.3 in July and September 2006).

On the basis of the N:P ratios, the P concentrations in the plant tissues and the lack of biomass response to P addition, P limitation can be ruled out (Güsewell & Koerselman, 2002). This suggests that the critical N:K ratio indicating possible K limitation or co-limitation of K and N lies at a higher level than is currently assumed (2.1 g g^{-1}, Olde Venterink *et al.* 2003). Our results suggest that the critical N:K ratio is situated around 4 g g^{-1}, at least for this specific hay meadow plant community.

Dilution effect

The tissue nutrient concentrations clearly show the effect of dilution by growth. Concentrations of N were lower in the +PK, +NK (HDD) and +NPK treatments than in the +NP and unfertilised (HDD) treatments (Figure 2a; Table 5). Biomass growth in this period was stimulated by added N and on HDD sods also by added K. In spite of the addition of N in the +NK and +NPK treatments on HDD sods, N concentrations were lower than in the control treatment. Although the total N-pool in the aboveground biomass was larger in these treatments, N concentrations were lower, because N was distributed over a larger total biomass ('dilution'). Although neither the +PK nor the control treatment received any N, the concentration of N in the nutrient tissue on HDD sods was nevertheless lower in the +PK treatment, because the total biomass was larger than in the control treatment, showing the limiting or co-limiting effect of K in this soil. Despite the addition of N in the +NPK treatment, N concentrations in this treatment were lower than those in the +NP treatment, because the +NPK treatment stimulated biomass production by the addition of K.

The same dilution effects were seen in the P concentrations in the vegetation on sods from both ZWS and HDD (Figures 2c and 2d; Table 5). The +NK treatment yielded a lower tissue P concentration than the control treatment, because the stimulation of biomass production by N in the +NK treatment caused dilution of P. The other treatments that increased biomass production had higher P concentrations, because they received P with the fertilisation. On HDD sods, however, the +NP treatment led to a higher tissue P concentration than the +NPK treatment, probably because biomass production in the +NP treatment was limited or co-limited by K. Similarly, the limitation by N on ZWS sods is apparent from the higher concentrations of P in the biomass of the +PK treatment compared to the +NPK treatment. The concentrations of K also show a clear dilution effect (Figures 2e and 2f; Table 5). Concentrations of K were smaller in the +NK (HDD) and +NPK treatments than in the +PK treatment, because the latter stimulated biomass production less.

These results imply that when a nutrient is added with fertilisation, this will not necessarily increase the concentrations of this nutrient in the plant tissue; in fact, concentrations of the nutrient can even be lowered by the addition. Moreover, the concentration of a nutrient added with the fertiliser seems to be increased if the limiting or co-limiting nutrient is not added. This dilution effect is not often shown in other experiments, due to high nutrient gifts leading to luxury consumption, which are often not corresponding with the field situation. At species level, a drop in the concentration of an added nutrient was also observed by Güsewell & Koerselman (2002). This may be an important issue in relation to the decomposibility of the plant material (Aerts & Chapin, 2000). In many cases, decomposibility is determined by the C:N ratio of the litter (e.g. Aerts & Chapin, 2000). It has frequently been hypothesised that plant material from nutrient-rich sites decomposes faster because of a lower C:N ratio in the plant litter (Vitousek, 1982; Aerts & Chapin, 2000). Aerts *et al.* (1995) showed that in an N-limited peatland dominated by *Carex acutiformis*, N concentrations in the plant tissue remained the same after N fertilisation. Our results, however, show that it is even possible that fertilisation leads to higher C:N ratios in the vegetation tissue, depending on whether N is still limiting biomass production.

Nutrient availability

Since nutrient availability is not only determined by the nutrient concentrations in the soil, but also by nutrient mobility and by the ecophysiology of the plant species (including mycorrhizae) involved (e.g. Aerts & Chapin, 2000; Lambers *et al.,* 2006; Chapin *et al.,* 1986a; Olff & Pegtel, 1994), differences in the concentrations of particular nutrients in the soil cannot be used to estimate the type of nutrient limitation. Organic matter forms a large pool of N, which can become available for uptake by plants through mineralisation. Therefore, concentrations of total N and of the directly available inorganic N (nitrate and ammonium) may both be worse predictors of the availability of N than decomposition rates (Chapin *et al.,* 1986b; Olde Venterink *et al.,* 2002). This means that the N concentrations measured in the soil (Table 2) do not provide information on the actual N limitation of the vegetation. For P, the soil-bound fraction forms the largest pool. P is effectively bound to Fe and Al (hydr)oxides and to Ca in the soil (Patrick & Khalid, 1974; Goldberg & Sposito 1984; Golterman 1995). Desorption can take place by several processes, including changes in redox potential and pH. The HDD and ZWS soils are both very rich in iron, which regulates the concentrations of o-PO_4 in the porewater to a great extent (Loeb *et al.,* in press; Loeb *et al.,* 2007). Upon flooding, these soils become anoxic and the reduction of iron leads to higher concentrations of o-PO_4 in the pore water. In our experiment, however, no flooding took place and the sods were kept oxic. In this situation, pore water concentrations will be low and

iron(hydr)oxides will bind o-PO_4 tightly, although part of this fraction is still available for plants due to the exudation of acids and phytochelatins, and the action of mycorrhizae (Aerts & Chapin, 2000). Concentrations of P in the plant tissue were significantly higher on sods from both locations in the treatments receiving P. This probably means that the vegetation effectively took up the supplied PO_4^{3-} before it was adsorbed and ended up as a small part of the total P pool in the soil. K, on the other hand, may show a stronger relationship between soil concentrations and uptake. Schaffers (2002) found a close correlation between K^+ extracted in a weak extraction with 0.01 M $CaCl_2$ and K uptake by vegetation. Soil samples taken from the HDD site showed a much lower concentration of K than soil samples from the ZWS sites. These lower concentrations may therefore explain the development of K limitation in the vegetation on sods from HDD, whereas this was not the case in the vegetation on the sods from ZWS.

Effects on family abundance

Our results show a differential response of plant families to the fertilisation treatments. The *Poaceae* performed worse in the +PK treatment than in the treatments in which N was added. This response of grass species to N fertilisation has been reported before (Berendse, 1990; Bobbink, 1991; Güsewell, 2004). On the sods of HDD, *Brassicaceae* species performed better under +NP fertilisation than in the +NPK treatment, whereas *Ranunculaceae* species performed worse in this treatment. We expected to find effects on the *Fabaceae*, which are able to fix N_2 from the air and thus have a competitive advantage under fertilisation without N (Bobbink, 1991). The absence of any significant effects on this family might be due to the small and scattered presence of members of this family on our sods. Summarising, fertilisation may indeed lead to shifts in the abundance of different families, and it is not only the actual load of each nutrient but also the ratio between the different nutrient loads that will affect species composition.

Consequences for the field situation

The experiment shows that nitrogen is limiting biomass production at both locations. Hence, nitrogen could still be important in the regulation of biomass production in biodiverse grasslands along N-polluted rivers. Yet, it should be noted that the ratio between nutrients in the river sediment deposits may also influence the type of limitation (Olde Venterink *et al.*, 2006). High nitrogen loads during flooding by nitrogen-rich rivers (such as the one in our study) may lead to increased growth of grasses at the expense of other species. In-

creased growth of grasses might therefore be expected to occur in the future, as a result of more frequent floods due to global climate change. In areas with a high atmospheric load of nitrogen, including the Netherlands, atmospheric deposition may induce similar effects.

In the vegetation from HDD, co-limitation by K developed after a year in which only N limitation was found. It can of course be questioned whether this K limitation could also develop in the field, as in the greenhouse this limitation could have developed due to clipping and leaching of K from the soil. Although this may be true to some extent, the development of the K limitation or co-limitation in our experiment may well indicate that this development may also occur in the field at HDD, as a result of hay-making or leaching of K due to drainage, or as a result of increased supply of N, which is the first limiting nutrient. In addition, a study by Schaffers (2000) of well-developed and fragmentary *Fritillario-Alopecuretum pratensis* plant communities along roadsides found that the well-developed *Fritillario-Alopecuretum pratensis* plant communities might be co-limited by K and N and the fragmentary ones by N and P. Although this study, as well as the present one, were conducted on limited field sites, both indicate that the K-poor conditions of the soil and the possible K limitation or co-limitation of the vegetation might be necessary conditions for the development and maintenance of well-developed *Fritillario-Alopecuretum pratensis* plant communties. The finding of K co-limitation is also important in general, as K-limitation is not frequently studied and reported in literature.

We conclude that for both hay meadows, an additional high supply of nutrients (N and to some extent also K) may be harmful to the survival of the present rare plant communities. This means that river water quality and river sediment quality may still be very important factors in the conservation of species-rich floodplain plant communities along eutrophic rivers worldwide. River management should therefore not only focus on the role of floodplains in removing nutrients from the river water, such as by nutrient sedimentation and denitrification, but also on the consequences of these functions for local biodiversity.

Acknowledgements

We would like to thank Sjaak Weenink, Ine Hendriks, Jelle Eygensteyn, Germa Verheggen, Gerard van de Weerden, Yvette Evers, Martin Versteeg, Denise Buijk and Janneke Roelofs for their help in taking and processing the samples, and Jeroen Breedenbeek (State Forestry Service) for his kind permission to collect the sods. This study was financially supported by the Netherlands Organisation for Scientific Research (NWO) as a part of the TRIAS programme (project 835.80.010).

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7

Synthesis: Biogeochemical processes influencing eutrophication and toxicity in riverine wetlands

Introduction

As described in Chapter 1, river ecosystems in the Netherlands have changed enormously in the last thousand years. Since the Early Middle Ages rivers have been subjected to endikement, flow regulation and fragmentation (Van Urk & Smit, 1989; Wolf *et al.*, 2001). Original forests have been cut down and the floodplains were transformed into agricultural pastures, cropland and hay meadows. Backswamps were cut-off from the floodplains by dikes and were drained and transformed into agricultural land as well. More recently, rivers were disconnected from the sea in the intertidal area, by the Delta Plan and the construction of the Afsluitdijk (Smits *et al.*, 2006). Meanwhile, river water quality changed as the rivers were considered the most convenient medium for the disposal of domestic and industrial waste, leading to a dramatically bad water quality during the first half of the 1970s (Van der Weijden & Middelburg, 1989; Van den Brink *et al.*, 1991;Van den Brink, 1994). Since then, major improvements in water quality have been achieved, but concentrations of nitrate, sulphate and chloride in the river water remain elevated today.

At present, we may find ourselves at a turning point in the history of river management. The extremely high waters in the rivers Rhine and Meuse in 1993 and 1995 have led to the understanding that floods might not be prevented by the raising of the river dikes solely, especially as global climate change is expected to increase the risks of floods in future. In the Dutch Space for the River Plan (Ministery of Transport, Public Works and Water, 2000) flood prevention is accompanied by the lowering of floodplains, the removal of groins and summer dikes and the establishment of water storage areas, all enlarging the connectivity of the river with its floodplains. Substantial quantities of agricultural land in the floodplains are transformed into nature reserves. In the former intertidal area, alterations have also been planned, although they may not be as rigorous as the changes proposed in the Space for the River Plan. The deterioration of the ecosystems in Lake Haringvliet (Smits *et al.*, 2006) and Lake Grevelingen (Nienhuis, 2006) has raised the awareness of the effects provoked by the closure of these, now stagnant, water bodies from the sea. For this reason, the sluices closing off the Oosterschelde have a more flexible design. Also for the Haringvliet sluices partly reopening is planned for (Paalvast *et al.*, 1998).

The increasing connectivity of the floodplains with the river water and the departure of agriculture from the floodplains creates ecological opportunities, but might also increase negative interactions between river water and soil quality for the development of target plant communities. The high concentrations of phosphate in the floodplain soils, mainly caused by the use of fertilisers, and the high sulphate concentrations in the river water may cause internal eutrophication when redox potential drops during inundation, and potentially phytotoxic hydrogen sulphides might be formed. Additionally, also the high concentrations of nitrate and chloride in the river water may enhance eutrophication and toxicity. Alterations in hydrological regimes, such as the re-establishment of the tide in Lake Haringvliet and more frequently occuring summer floods, expected as a result of global climate change, may also have effects on these processes. The present thesis examines the biogeochemical processes influencing eutrophication and toxicity in riverine floodplains in the Netherlands. This research especially focusses on the effects of the changes in water quality, in particular the high sulphate concentrations, and in soil quality on vegetation development in the scope of the present and future river management policies and expected global climate change.

Eutrophication

Under aerobic circumstances phosphate in floodplains is bound to iron, to iron and aluminium (hydr)oxides and to calcium and calcium carbonate. When flooded, oxygen is depleted by the decomposition of organic matter and, sub-

sequently, other electron acceptors are used for oxidation processes. One of these alternative electron acceptors is Fe^{3+}. When Fe^{3+} is reduced to Fe^{2+}, affinity for phosphate binding decreases, and part of the phosphates adsorbed to the iron and iron(hydr)oxides is released to the pore water (Figure 1) (**Chapters 2**,**3**,**4** and **5**; Patrick & Khalid, 1974; Ponnamperuma, 1984). This can result in an increase of phosphate concentrations in the pore water, up to 90 times the initial concentration (**Chapter 4**). Naturally, floodplain soils containing clay are rich in iron. Extra iron can be supplied to the floodplains by the discharge of iron rich groundwater (e.g. as was the case for the alder carr soil described in **Chapter 3** and for the hay meadows in **Chapters 2** and **6**). Whether reduction of iron will take place after inundation depends on the presence of easily degradable organic matter (**Chapter 3**). Thus, completely mineral soils, such

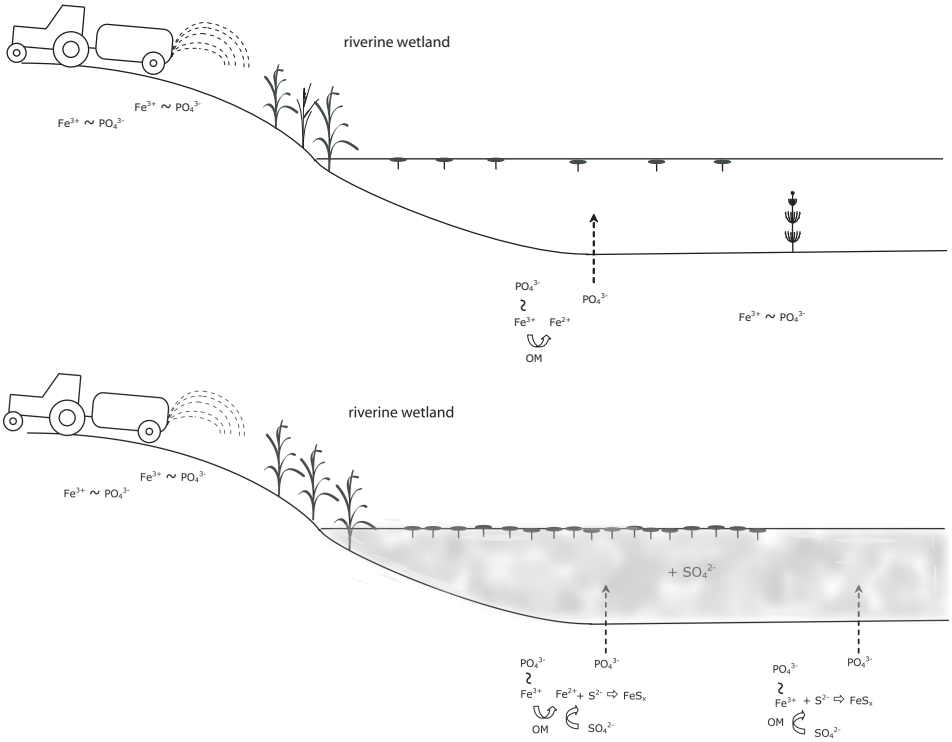

Figure 1. On soils with high concentrations of phosphate in comparison with iron, inundation leads to mobilisation of phosphate to the pore water and possible release to the surface water, leading to eutrophication (figure above). On the same soil, inundation with sulphate rich water may induce the release of extra phosphate (figure below). As long as iron is present in excess, sulphate reduction does not lead to the formation of phytotoxic dissolved sulphide species. OM = organic matter.

as those in clay and sand sedimentation areas where the topsoil has been removed, are not likely to release large quantities of phosphate in the short term. However, this may change as soon as some soil organic matter accumulates. Also nitrate could have an adverse effect on the release of phosphate, because it can be used by micro-organisms as an alternative electron acceptor in the oxidation of organic matter at a higher redox potential than Fe^{3+}. For this reason, nitrate may function as a redox buffer preventing the reduction of Fe^{3+} (Lucassen *et al.*, 2004).

Chapter 4 explores how the mobilisation of phosporus at inundation, mainly caused by the reduction of iron, could be predicted. It was hypothesized that floodplain soils that were more intensively used by agriculture would release more phosphorus, because these soils were assumed to have higher soil phosphorus concentrations and hence higher concentrations of iron bound phosphorus, susceptible to release to the pore water if iron would be reduced. However, this release appeared not to be directly related to the concentration of phosphorus bound to iron. It depends much stonger on the ratio between iron bound phosphorus and the total amount of amorphous iron in the soil (Figures 1 and 2). In other words, in two floodplains with the same load of phosphorus, having a similar iron bound phosphorus concentration, the release of phosphorus to the soil pore water by iron reduction will be different if they have different amorphous iron concentrations (**Chapter 4**; Zak & Gelbrecht, 2007). As the concentration of iron bound phosphorus and the concentration of amorphous iron are easily measurable soil characteristics, this ratio could be used to compare locations for their suitability for the construction of new wetlands or water storage areas.

It is known that sulphate reduction and subsequent competition between sulphide and phosphate for binding to iron is one of the main processes causing eutrophication in peatlands receiving allochtonous sulphate-rich water (Smolders & Roelofs, 1995; Smolders *et al.*, 1996; Lamers *et al.*, 2002). In **Chapter 3** we showed that in riverine floodplain soils sulphate reduction may also lead to an additional release of phosphate (Figure 1). However, this additional release is much smaller than the release caused by the reduction of iron that takes place upon flooding. Conversely, this additional release caused by the formation of sulphide was not detected at all in a number of experiments (**Chapters 2**,**3** and **5**). Obviously, floodplain soils differ from peatlands in their organic matter content, sometimes to an extent in which organic matter may become limiting for the reduction of iron and sulphate (**Chapter 3**). Yet, more importantly, do they differ from peatlands in iron concentrations. I therefore hypothesize that the possibly lower phosphate saturation in floodplain soil, caused by the high iron concentrations, prevents the competition between sulphide and phosphate (Figure 2) (**Chapter 2**; Zak *et al.*, 2006).

Surprisingly, the high sulphate concentrations in oligohaline water envisaged when the Haringvliet will be reconnected with the sea (concentrations about 4.5 times higher than in fresh river water), do not cause any additional phosphate release to the pore water in the root zone by sulphide formation either (**Chapter 5**). Moreover, the restored tidal movement increases the redox potential by the stimulation of oxygen intrusion into the soil, resulting in less iron reduction and concurrent phosphate mobilisation. Canavan (2006), however, showed that the higher sulphate concentrations correlating with the restoration of the Haringvliet, are expected to cause an extra release of P in the sediments of

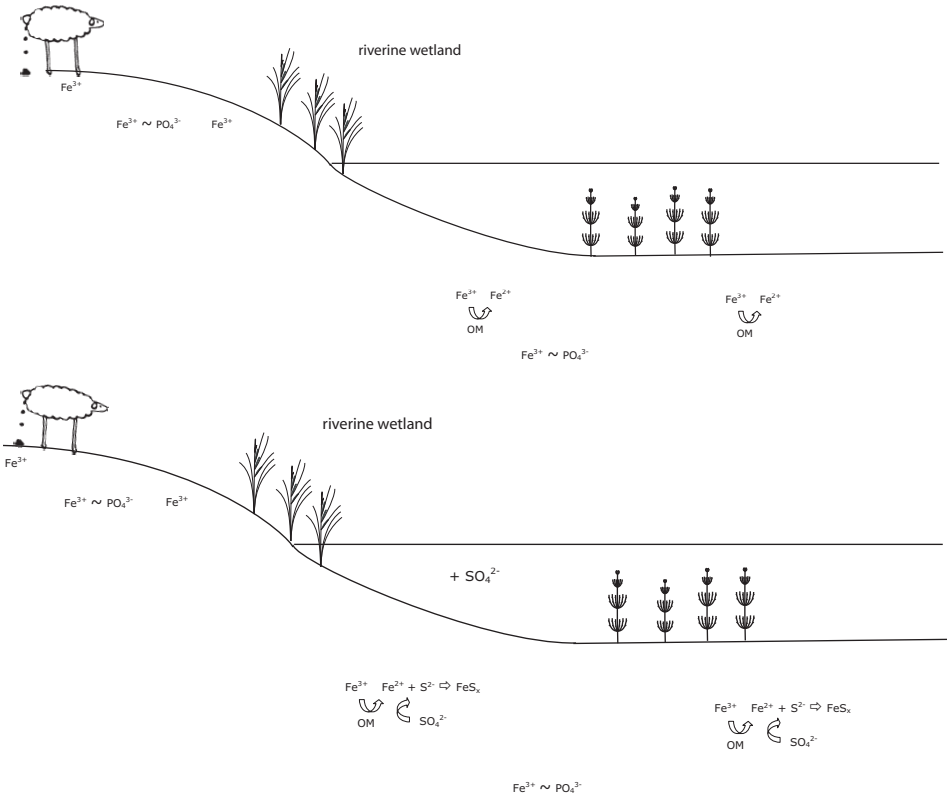

Figure 2. On soils with low concentrations of phosphate in comparison with iron, inundation leads to less mobilisation of phosphate to the pore water and concomitant release to the surface water than in soils with high concentrations of phosphate compared to iron (figure above; Figure 1). Inundation with sulphate rich water does not cause any additional release of phosphate (figure below). As long as iron is present in excess, sulphate reduction does not lead to the formation of phytotoxic dissolved sulphide species. OM = organic matter.

the bottom of Lake Haringvliet, which have a (more permanently) lower redox potential than the root zone of the wetlands from the shores of lake Haringvliet which we studied. This higher P release caused by the reduction of the extra sulphate, as wel as by an increase of bioturbative mixing (Canavan, 2006) is expected to cause higher P concentrations in the surface water. Additionally, the re-establishment of the tidal movement causes a higher exchange between pore water and surface water, resulting in higher phosphate concentrations in the surface water (**Chapter 5**). Thus, although the increase in sulphate concentrations will not cause an extra release of P in the root zone and the enlarged tidal amplitude may even induce a higher retention of P by the oxidation of iron, the expected higher sulphate concentrations may still have an effect on tidal wetlands via increased flooding with phosphate-rich surface water. In the soil pore water of the Haringvliet wetland mesocosm experiment no evidence was found for an increase in phosphate mobilisation by anion exchange with the higher chloride concentrations in the oligohaline water (approximatley 18 times higher than in fresh water) (**Chapter 5**), as has been supposed for peatlands (Beltman *et al.*, 2000).

Phosphorus is an essential plant nutrient. Some floodplain plant communities have been shown to be co-limited by P with respect to biomass production (Van Oorschot *et al.*, 1998; Beltman *et al.*, 2007), which means that an increase in availability of phosphorus may cause eutrophication effects, such as the dominance of fast growing plant species over others. But even if the plant community growing on a site where phosphorus is released, is not limited by phosphorus itself, the enhanced mobility of phosphorus could cause a release of phosphate from the floodplain soil to the surface water and may in that way result in eutrophication of plant communities within the same or in other floodplains. Many aquatic communities are known to be limited by phosphorus (Schindler, 1977; Hecky *et al.*, 1988; Moss *et al.*, 2005). Addition of phosphorus may thus lead to algal blooms in floodplain lakes.

Nitrogen is often the limiting factor in terrestrial biomass growth in (undisturbed) floodplains (Antheunisse *et al.*, 2006; Beltman *et al.*, 2007). In other words, the high input of nitrate from the river water into floodplains may favour fast growing plant species above the often more desired plant species of more nutrient poor habitats. The input of nitrogen does not necessarily occur through flooding with enriched river water; also sediment deposition (Antheunisse, 2006) and supply via groundwater contact with the river water will contribute to this input. In **Chapter 6** I showed that nitrogen, together with potassium, is the limiting nutrient for biomass production in the rare plant community *Fritillaria-Alopecuretum* in floodplains of the river Overijsselse Vecht. Here, input of nitrogen did not only lead to a higher biomass but also to

a higher dominance of grasses, which may threaten the survival of some of the original, characteristic, plant species.

Toxicity

Although iron is an essential nutrient for plants, high concentrations of iron can be toxic for plants (Snowden & Wheeler, 1993; Lucassen *et al.*, 2000). Ferric (oxidised) iron (Fe^{3+}) is highly immobile and therefore less available for plants than its reduced form (ferrous iron, Fe^{2+}). Reduction of iron by anaerobic respiration in floodplain soils, which are rich in iron naturally, can lead to toxicity for plants. Floodplain soils may release very high amounts of ferrous iron to the soil pore water when they are inundated (**Chapters 2** and **3**). The concentrations measured in the pore water of these sediments were in the same

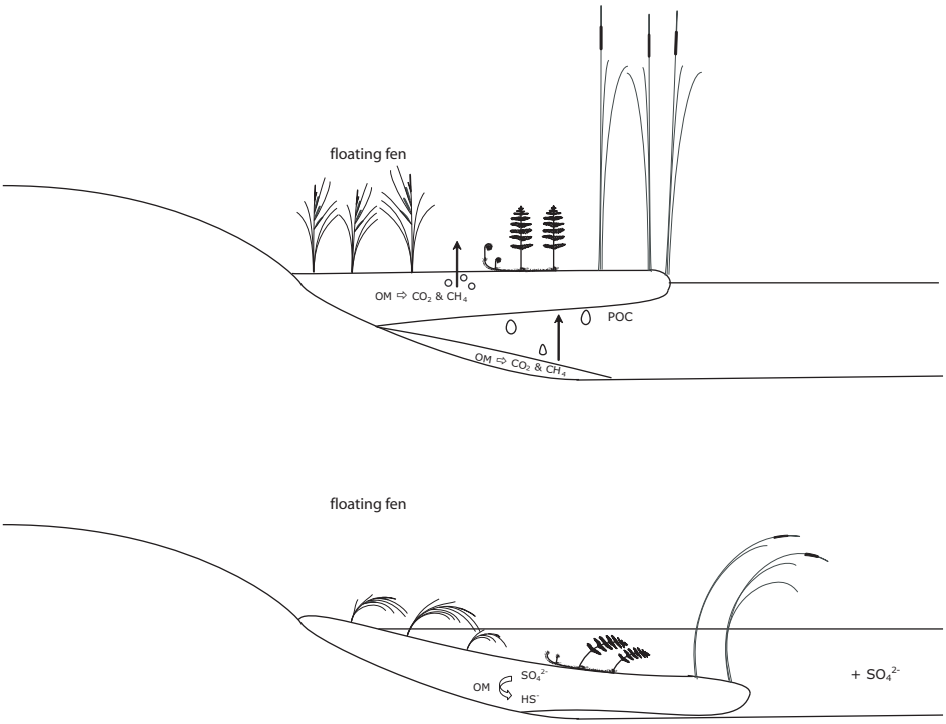

Figure 3. Floating fens depend primarily on the formation of methane for buoyancy (figure above). Inundation with sulphate rich water hampers methanogenesis. Additionally dissolved (hydrogen) sulphide is formed, which may be toxic for plants (figure below). OM = organic matter, POC = particulate organic matter.

order of magnitude as concentrations reported to be toxic for plants (Lucassen *et al.*, 2000; Van der Welle *et al.*, 2007). As the increase in the availability of nutrients without external supply has been described as 'internal eutrophication' (Roelofs, 1991), the production of toxic substances within a system, such as the conversion of ferric to ferrous iron, may in the same way be described as 'internal toxification'.

Although the word 'toxic' might be associated with unnatural and undesirable situations, the presence of high iron concentrations in wetland soils rich in iron is natural and may act as a selection criterion for plants species composition. Snowden & Wheeler (1993) showed that wetland plants (especially monocotyledon species) have a high tolerance for iron. For this reason they are better competitors at high iron concentrations than plant species of dryer habitats. Despite this high tolerance of wetland monocotyledons in general, we found that the wetland grass *Glyceria maxima* showed reduced photosynthesis at high iron concentrations, which could not be attributed to lower phosphate availability (Figure 4). Also among aquatic species high iron concentrations can lead to a shift in competitive strength, as was shown by Van der Welle *et al.* (2007) for *Elodea nuttallii* and *Stratiotes aloides* in enclosures in the field treated with and without iron. Next to nutrient availability, changes in hydrological conditions leading to alterations of iron availability might therefore also play a role in determining plant species composition in floodplains. This implies that the reduction of groundwater discharge due to drainage and to subsidence

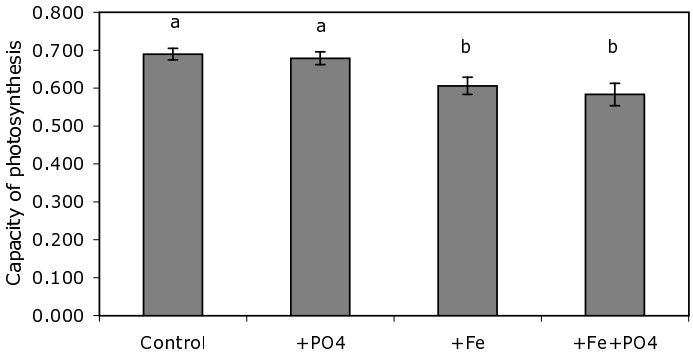

Figure 4. Capacity of photosynthesis (in relation to the maximum yield) of *Glyceria maxima* leaves. Measurements with a Diving Pulse Amplitude Modulation (Heinz Walz GmbH) took place after 25 days of treatment. Pore water iron concentrations were 1415 μmol L^{-1} on average for the +Fe and +Fe+PO_4 treatments and 145 μmol L^{-1} for the control and +PO_4 treatment. Error bars represent standard errors of the mean (n=6). Different letters indicate significant differences ($p < 0.05$) between treatments (Lammers, 2004; Lammers & Loeb, unpublished results).

of the land behind the dikes, may reduce the internal toxification, affecting species composition.

Sulphate reduction is a common process in inundated floodplain soils (**Chapters 2**, **3** and **5**; Miletto, 2007). This process, however, only leads to the presence of dissolved sulphide species (S^{2-}, HS^- and H_2S) if the soil concerned has a very low concentration of iron (Figure 3) (**Chapter 3**). In the presence of iron, sulphide will precipitate as FeS_x. In this way the presence of iron will prevent the formation of this potential phytotoxin, as was shown for common floodplain soils high in iron (Figures 1 and 2) (**Chapters 2**, **3** and **5**). In **Chapter 3** it was shown that in a peaty floating fen soil, containing only a small amount of iron, reduction of sulphate present in concentrations common in river water, led to toxic effects to the phytometer *Glyceria maxima*, due to the accumulation of hydrogen sulphide.

Next to those of sulphate and nitrate, concentrations of chloride are also still elevated in the river water. In the former brackish tidal areas, however, chloride concentrations are much lower than before the closing of the Haringvliet (approximately 2 mmol L^{-1} and 37 mmol L^{-1}, respectively). However, chloride plays, as a toxicant, a vital role for the maintainance of brackish water plant communities. The desalinisation of the former brackish tidal area caused the disappearence of several characteristic plants adapted to the salinity of the water, such as *Cochlearea officinalis* (Paalvast *et al.*, 1998). The restoration of the oligohaline conditions in lake Haringvliet will rehabilitate chloride toxicity, as was shown by the reduction of biomass production of the vegetation as well as by the higher internal free amino-acid concentrations in *Agrostis stolonifera* (**Chapter 5**). Although these effects are already known of euhaline environments, they had never been studied in oligohaline environments before. As we already observed the toxicity of chloride in such a short period of time, we believe that the restoration of oligohaline conditions in lake Haringvliet will ultimately lead to the re-establishment of plant species adapted to high chloride concentrations.

Although chloride toxicity will help in the restoration of oligohaline plant communities in the Haringvliet area, the high chloride concentrations found in the freshwater range of the river water can cause deterioration of freshwater riverine habitats. Van den Brink *et al.* (1991) and Van den Brink and van der Velde (1993) showed that the increased salinity of the river water at levels of 7 mmol L^{-1} may have caused a decline in the presence of freshwater macrophytes in floodplain lakes in the Netherlandsin the 1970s and 1980s.

Implications for nature management in riverine areas

This thesis stresses the importance of river water quality in the development of characteristic plant communties in river floodplains. The high nitrate concentrations in the river water may form a threat for eutrophication of some species rich plant communities (**Chapter 6**), whereas the high sulphate concentrations may cause the release of extra phosphate in floodplains with a high organic matter content and a high phosphate saturation (**Chapter 3**). Besides, both sulphate and nitrate can inhibit the production of methane in floating fen soils (Figure 3) (**Chapter 3**; Cappenberg 1974; Scholten & Stams 1995), which are, nowadays, rare in river floodplains, but very valuable from a nature conservation perspective. In this way they can sink floating rafts and hamper the succesion of open water to fen systems. On soils containing little iron, sulphate can be transformed into the phytotoxic hydrogen sulphide. The high chloride concentrations may also have negative effects on some plant species in floodplains (Van den Brink *et al.*, 1991; Van den Brink & Van der Velde, 1993).
Notwithstanding these effects, sulphate concentration does not play any role in the Dutch elaboration for river water quality of the European Union Water Framework Directive, the most important directive for water quality at this moment. Chloride concentrations must not exceed 4.2 mmol L^{-1}, a 20% increase of the current concentration. Moreover, nitrogen in the river water is considered only important for its effects on phytoplankton growth in estuaries, as phosphate is expected to limit biomass production in rivers (Van der Molen & Pot (eds), 2007). The possible effects of river water nitrate on floodplain plant communities have not been taken into account. At the moment, maximum concentrations for nitrogen and phosphorus in the river water in the Water Framework Directive are set at 0.18 mmol L^{-1} for total N and 4.5 µmol L^{-1} for total P. For N the optimal situation, which should be derived from the concentration in the natural reference situation, is set at 0.14 mmol L^{-1}, which is far above the estimated historical background concentration of 0.02 mmol L^{-1} for nitrate (Van der Weijden & Middelburg, 1989). The results presented in this thesis call for stricter standards and the incorporation of sulphate in the Water Framework Directive.

Not only water quality is important for processes influencing toxicity and eutrophication, soil properties are at least equally important. The reduction of iron in floodplain soils causes a release of phosphate. This release appeared to be much larger than the release that was observed due to sulphide formation (**Chapters 3**, **4** and **5**). As a result of this process, floodplain soils may act as a source rather than a sink for phosphorus when they are inundated. Because in many floodplains in the Netherlands high amounts of fertiliser have been used, not all locations will be suitable for the creation of new floodplain wetlands regarding internal eutrophication. Their suitability will depend, among other

facors, on the iron-bound-phosphorus to amorphous iron ratio, a measure for their phosphate saturation (**Chapter 4**). Top layer removal, which is a common measure in other landscapes in the transformation of agricultural land into nature reserves, may not be as effective in floodplains. Whereas in agricultural soils outside the floodplains a destinct phosphate profile has formed, such a profile may be less apparent in floodplains due to sedimentation or erosion. It is therefore important that possible locations for river wetland rehabilitation or for flood water storage should be compared for their soil characteristics, especially their iron-bound-phosphorus to iron ratio.

Global climate change is expected to increase both temperature and the risk of summer floods (Christensen & Christensen, 2003). This implicates that floods may occur at higher temperatures. In **Chapter 2** we showed that anaerobic decomposition of organic matter, which takes place upon flooding, is much faster at higher temperatures. This means a faster reduction of iron, a faster release of phosphate, and a faster transformation of sulphate into sulphide. In this way, global climate change may intensify eutrophication and phytotoxicity. In addition, the effects of flooding might be more severe for ecophysiological reasons when flooding takes place during the growing season of plants (Van Eck *et al.*, 2004; 2006; Koutecky & Prach, 2005).

In river management, the former floodplains, which are now cut-off by the dikes, are often overlooked. Here, old river meanders (oxbow lakes), sporadically still featuring *Stratiotes aloides*, alder carrs and shallow clay and loam excavations supporting hay meadows rich in orchids, can be found. At these less dynamic locations with little water table movement, floating fen formation could still take place, if water quality allows so (**Chapter 3**). Although these habitats are no longer in contact with the river (except for places receiving river water via groundwater discharge) they actually would not exist if they had not been shaped by the river. Today, formation of new oxbow lakes and floodplain swamps and marshland far away from the river bed would be inconceivable, because of safety reasons. Therefore, to my opinion, there is a strong demand for protection of these remainig areas. It would be regrettable if these areas would be sacrificed to intensive agriculture in exchange for the departure of agriculture from the floodplains within the winter river beds, as has been proposed by De Bruin *et al.* (1987).

In most of the newly developed nature reserves in the riverine floodplains the dominant management strategy is based on the stimulation of geomorphological processes and extensive grazing by large herbivores. The pre-historical situation functions here as a reference situation. The chosen references and management form a sharp contrast with those chosen frequently in other landscapes. For heathlands, fens and grasslands along smaller streams the

focuss is often on so called 'semi-natural' landscape types; here, the vegetation is determined by the traditional, historical landuse of, for instance, mowing, grazing or reed cultivation, which often resulted in a high biodiversity. In the river floodplains some small reserves where these kinds of traditional management take place, still maintain. The *Fritillario-Alopecuretum* plant community, described in Chapters 2 and 6, is an example of such a meadow that is traditionally mown. Also other plant communities belonging to the *Arrhenatheretalia*, which are grazed or mown (Zuidhoff *et al.*, 1996), were characteristic for the less dynamic places in floodplains and on dikes along the Dutch rivers before the intensivation of agriculture. The importance of these meadows in a European perspective has recentely been recognised in the European Habitat Directive (Kiwa & EGG, 2006; Weeda *et al.*, 2007). To my opinion, important profit could be made if such management strategies would be applied to a larger part of the former, intensively used, agricultural land, in addition to the reserves where wilderness is used as a reference. This could promote both biodiversity and cultural heritage.

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Summary

River ecosystems are among the most impacted ecosystems around the world. In the Netherlands, major anthropogenic changes in the river systems have occurred since the Early Middle Ages. Dikes cut off the rivers from their original floodplains and human, industrial and agricultural waste changed the chemical composition of the river water significantly. Although river water quality improved since the 1970s, concentrations of nitrate, sulfate and chloride are still strongly elevated in the river water. Moreover, floodplains themselves have been subjected to intensive fertilisation as a result of agricultural management. New river safety strategies enabled the conversion of agricultural land in the floodplains into nature reserves. It was, however, uncertain whether the biogeochemical interactions between the polluted river water and the polluted soils would still meet the demands of certain characteristic vegetation types. In this thesis the emphasis is in particular on the influence of these biogeochemical processes with respect to eutrophication and phytotoxicity.

In **Chapter 2** the effects of flooding at low and high temperatures are addressed. Flooding leads to a switch to anaerobic soil respiration in riparian meadows along rivers, causing iron reduction and a corresponding release of phosphate. In addition, pollution of river water with sulphate may lead to higher phosphate release as a result of sulphide-iron interactions. As global climate change is expected to increase both temperatures and the risk of summer flooding, floods may occur at higher temperatures, leading to faster anaerobic processes in soils. In a mesocosm experiment we tested the effects of flooding with sulphate-polluted river water at two temperatures on sods from a riverine hay meadow. Redox potential dropped during flooding, leading to higher concentrations of Mn^{2+}, Fe^{2+}, PO_4^{3-}, NH_4^+ and Ca^{2+} and a higher alkalinity of the soil pore water. Upon desiccation, redox potential increased immediately, leading to the oxidation of Mn^{2+}, NH_4^+ and Fe^{2+} and causing immobilisation of PO_4^{3-} and a temporary drop in pH. Inundation at 20°C resulted in a much faster release of Mn^{2+}, Fe^{2+}, PO_4^{3-} and Ca^{2+} and a higher acid consumption compared to flooding at 5°C. Reduction of the added sulphate did, however, not lead to additional mobilisation of phosphate through competition with the produced sulphide for binding to iron, because of the high iron concentration in the soil, which is a characteristic of many floodplain soils. It was concluded that seasonality of flooding determines accumulation rates of potential phytotoxins and the release rate of phosphate.

Although phosphate concentrations have been reduced, the rivers Meuse and Rhine are still polluted with sulphate, which most probably affects vegetation development in newly created riverine wetlands. In **Chapter 3** the influence

of flooding with river water rich in sulphate was tested on three soil types from floodplains of the river Meuse using flow-through and batch experiments. Soils were selected for contrasting concentrations of iron and organic matter and originated from a floating fen (iron-poor, organic), an alder carr (iron-rich, organic) and a clay pit (iron-rich, low in organic matter). Flooding induced mobilisation of phosphate. Sulphate only enhanced this effect in the alder carr soil, where sulphide and phosphate competed for binding to iron. Only in the floating fen soil did the addition of sulphate result in the formation of free sulphide, which reduced the growth of *Glyceria maxima*, serving as a phytometer. In addition, the floating soil started to sink, due to falling methane concentrations. In the different soil types methane production was hampered by the presence of more favourable electron acceptors such as sulphate in the water and iron(III) in the soil. It was concluded that the effects of inundation with sulphate-polluted water strongly depend on the soil type: under iron-poor circumstances, free sulphide may accumulate, leading to phytotoxicity, while in soils rich in iron, sulphide toxicity is prevented, but phosphate availability may be increased. In addition, shortage of easily degradable organic matter can limit the formation of potential toxicants such as ammonium, iron and sulphide.

As shown in **Chapters 2** and **3**, iron reduction in riverine wetlands may cause the release of large quantities of phosphorus upon inundation. As phosphorus is an important nutrient causing eutrophication in aquatic systems, it is important to have a tool to predict its potential release. In **Chapter 4** we examined the P release to the soil pore water in soil cores from floodplains in the Netherlands and from less anthropogenically influenced floodplains from Poland. During the inundation experiment, concentrations of P in the pore water rose to 2–90 times the initial concentrations. P release was, however, not directly related to the historical management (fertilisation history) of the soils. An important predictor variable of P release was found in the ratio between the concentration of iron-bound P and amorphous iron. This ratio may provide a practical tool for the selection of new areas for wetland creation, and for impact assessment of plans for riverine wetland restoration and flood water storage.

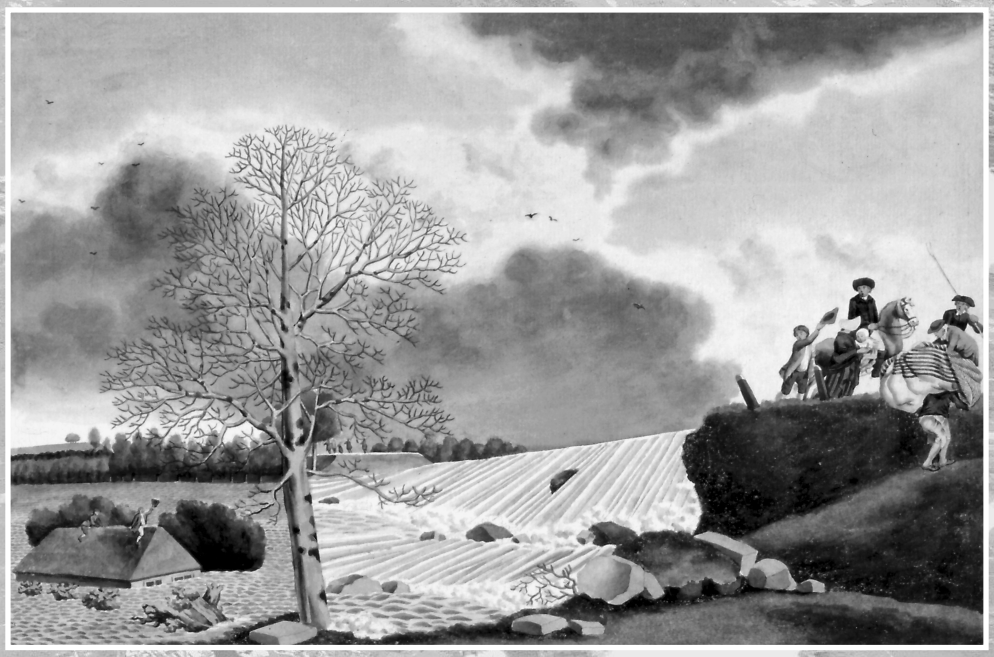
In 1970, most of the Rhine and Meuse estuary was closed off from the open sea by the Haringvliet sluice dam and changed into a stagnant freshwater basin. Currently, authorities are supporting the intention to partly reopen the sluices to regain part of the lost biodiversity. In **Chapter 5**, we examined the effects of the increased tidal amplitude and salinity associated with a partial reopening of the sluices on soil biogeochemistry and vegetation. Effects were studied in mesocosms using sods from a nature reserve and an agricultural area on the banks of the Haringvliet estuary. In a full factorial design, the mesocosms were exposed to a tidal amplitude of 0.2 m and a salinity of 3 ‰

(oligohaline) for one year. Pore water and surface water analyses revealed that the tidal regime enabled oxygen penetration into the soil, decreasing anaerobic processes including iron reduction, leading to an overall immobilisation of phosphate. The tides also decreased methanogenesis. The intrusion of oligohaline water led to increased cation exchange, resulting in higher concentrations of calcium in the pore water and leaching of this ion into the surface water. In the brackish treatments, biomass production was hampered by the increased salinity. Higher concentrations of asparagine, proline and serine in leaf tissue of *Agrostis stolonifera* also indicated increased salt stress in the brackish treatments. As a result, plant species adapted to oligohaline water are expected to show increased competitive strength, enabling the restoration of characteristic vegetation types in the field.

Flooding by nutrient-rich lowland rivers may lead to a high influx of nutrients into floodplains causing eutrophication and the dominance of fast-growing species in floodplain plant communities. It is, however, doubted whether the growth of eutrophic floodplain plant communities along impacted rivers is still limited by nutrients. In **Chapter 6** potential nutrient limitation and soil characteristics of the vegetation of two hay meadows, both belonging to the rare *Fritillario-Alopecuretum* plant community, in the floodplain of the eutrophic river Overijsselse Vecht, are studied. The meadows had different fertilisation histories: one was a species-rich hay meadow managed as a nature reserve, the other a newly created nature reserve that had been used as an agricultural pasture before. Sods collected from both locations were transferred to a greenhouse and fertilised weekly with N+P, N+K, P+K, N+P+K or control solutions, and harvested 3 times a year during 2 successive growing seasons. Biomass production of sods from both locations of this floodplain appeared to be still limited by N. Interestingly, the sods from the original nature reserve were also limited by K, but only in the second year. Fertilisation caused a shift in the relative abundance of the different plant families. Tissue nutrient concentrations were increased by fertilisation with the nutrient in question, but decreased if biomass production was stimulated. It was concluded that even in eutrophic river areas, the nutrient concentrations of the surface water may still determine the development of potentially biodiverse floodplain vegetation.

This thesis shows that both river water quality and sediment quality strongly determine ecosystem functioning and vegetation development. The high nitrate concentrations in the river water may cause eutrophication in some species rich plant communities, whereas the high sulphate concentrations may cause the release of extra phosphate in floodplains with a high organic matter content and a high phosphate saturation. Both sulphate and nitrate can inhibit the production of methane in floating fen soils. Notwithstanding these effects, sulphate concentration does not play any role in the Dutch elaboration

for river water quality of the European Union Water Framework Directive, and the standard for nitrogen is very high compared to the historical background concentration. Besides the effects of water quality, the reduction of iron in floodplain soils causes a release of phosphate. This release appeared to be much larger than the release that was observed due to sulphide formation and depends on the phosphate saturation. It is therefore important that possible locations for river wetland rehabilitation or for flood water storage should be compared for their soil characteristics, especially their iron-bound-phosphorus to iron ratio, before they are selected.



Nederlandse samenvatting
speciaal voor niet-ecologen

LAAG HANGT DE ZON. DE LANGE BOSSCHEN, DIJKEN
Van ondoorschijnendheid, weren de baren
Van 't roode licht, dat afdruipt van de blaren,
Doorsijp'lend, waar tot lek de twijgen wijken;

De vlakke stroomen, die 't doorzichtig strijken,
Kan niet het voorland, ruigbegroeid met varen,
Niet kan de takkenglooiing doen bedaren
De steile vloed, die heen spoelt over de eiken;

Over de kruin en –dijkbreuk– door de wanden
Stort zich de oranje branding op de landen,
Wijd vloeiend goud, als uit een fabelbron;

In 't oosten bouwt de nacht zijn wolkendammen;
Meezuigt de zon de vloed van koele vlammen,
En ze ebben weg onder de horizon.

JOHAN ANDREAS DÈR MOUW

Inleiding

Bovenstaand gedicht moest ik analyseren tijdens mijn mondeling tentamen Nederlands. Het was de winter van 1994/1995 en ik deed eindexamen VWO in Wageningen. Op de achtergrond speelden de hoge waterstanden van de rivieren en het gevaar voor een dijkbreuk. Klasgenoten uit de Betuwe waren geëvacueerd en leerlingen die in de lage delen van Wageningen woonden, hadden alvast hun koffers moeten pakken voor het geval ook zij moesten vertrekken. Onderweg naar school zag ik de evacués een droger heenkomen zoeken; auto's volgepropt met huisraad, sommige zelfs met canapé's op het dak gebonden. In totaal werden er in die periode ongeveer 250.000 mensen geëvacueerd. Uiteindelijk hebben de dijken het gehouden, maar het was kantje-boord.

Naar aanleiding van de hoogwaters van die winter en de winter ervoor werd besloten dat er drastische maatregelen nodig waren om het rivierengebied weer veiliger te maken. Deze maatregelen werden uitgewerkt in het plan 'Ruimte voor de Rivier'. Dit plan vormde een duidelijke breuk met het rivierbeleid dat er tot dan toe gevoerd werd. Het 'oude' rivierbeleid bestond uit het regelmatig ophogen van de dijken. Deze ophogingen waren nodig, omdat de uiterwaarden door opslibbing steeds hoger kwamen te liggen. Aan de andere kant van de dijk kwam het land echter steeds lager te liggen; door ontwatering klinken de klei en het veen in. Na het hoogwater van 1995 groeide het inzicht dat het ophogen van de dijken alléén geen duurzame oplossing zou vormen om het rivierengebied tegen overstromingen te blijven beschermen, zeker niet omdat de verwachting is dat hoogwaters door het versterkte broeikaseffect in de toekomst vaker voor zullen komen. De aanvullende maatregelen, die momenteel nog steeds in uitvoering zijn, bestaan onder andere uit het verlagen van uiterwaarden, het weghalen van zomerdijken en het landinwaarts verplaatsen van dijken op punten waar de rivieren smal zijn. Op die manier wordt er meer ruimte voor het water gecreëerd. Ook binnendijks worden er maatregelen genomen: er wordt gezocht naar gebieden die bij extreem hoogwater onder water gezet kunnen worden (waterberging) en naar manieren om regenwater langer vast te houden in het gebied waar het gevallen is (waterretentie), in plaats van het zo snel mogelijk naar de rivier af te voeren. De nieuwe maatregelen tussen de dijken zijn moeilijk te combineren met de landbouw, maar geven wel ruimte voor het ontwikkelen van nieuwe natuurgebieden.

Geschiedenis van het rivierengebied

Veel mensen ervaren het rivierenlandschap als 'natuur'. Toch is het rivierenlandschap misschien wel het vroegst beïnvloede en een van de sterkst door de mens veranderde landschappen van Nederland. Vóór de beïnvloeding door de mens, zo rond de Romeinse tijd, zag het rivierengebied er compleet anders uit. Het verschil tussen hoog- en laagwater was in die tijd veel kleiner, maar het gebied dat bij hoogwater overstroomde was veel groter. Tussen de rivieren lagen overstromingsvlaktes van tientallen kilometers breed. De rivieren hadden geen vaste loop, maar verlegden zich telkens naar het laagste punt in het landschap, waarbij ze oude rivierarmen als meertjes achterlieten. Er was veel bos met een meer kruidachtige begroeiing op natte plekken en plaatsen met een hoge dynamiek.
Vanaf de Vroege Middeleeuwen werd de beïnvloeding door de mens zichtbaar. In die periode werden de eerste dijken aangelegd. Deze dijken lagen niet om de rivier, zoals later gebruikelijk werd, maar om de steden en dorpen. Binnen een paar eeuwen werden echter ook de rivieren bedijkt. Rond 1450 was de bedijking van ongeveer alle grote Nederlandse rivieren voltooid. Hierdoor werd

het gebied dat met hoogwater overstroomde drastisch verkleind. In de 19e eeuw werden de rivierlopen rechtgetrokken en vastgelegd. Sindsdien zitten de rivieren vast in een 'keurslijf' dat weinig bewegingsvrijheid geeft.
Ook het landgebruik veranderde. Het bos werd gekapt voor het hout en later ook om ervoor te zorgen dat het water beter weg kon stromen tijdens hoogwater. Het maakte plaats voor landbouw: graslanden met koeien, dijken met schapen, hooilanden en akkers. Er werden fabriekjes opgericht die plaatselijk het opgeslibde klei weggroeven om er bakstenen van te maken.
Ook in de lage delen van Nederland, waar de rivieren door getijden beïnvloed werden, veranderde er veel. De eerste grote verandering vond plaats met de aanleg van de Afsluitdijk in 1932, die van de zoute Zuiderzee het zoete IJsselmeer maakte. Hierdoor verdween de invloed van getij en zout uit de IJssel. Een tweede keerpunt was de watersnoodramp die zich tijdens een stormvloed in 1953 voltrok. Tijdens deze ramp vielen er 1835 doden en een veelvoud ervan raakte zijn huis en vee kwijt. In de drie opvolgende decennia werd er hard gewerkt aan het Deltaplan, waarmee de invloed van de zee werd buitengesloten. In het kader van het Deltaplan werd in 1971 het Haringvliet afgesloten met de Haringvlietdam. De effecten voor de natuur waren desastreus, vooral voor de Biesbosch, een gebied dat vóór die tijd een van de grootste zoetwatergetijdengebied van Europa was. Eerst was het verschil tussen de waterstand bij vloed, wanneer het zoete rivierwater niet naar zee af kon stromen en dus opgestuwd werd, en de waterstand bij eb twee meter. Sinds de afsluiting van het Haringvliet is hier nog maar 30 centimeter van over. Het gebied dat eens begroeid was met biezen en de zeldzame Spindotter is hierdoor veel kleiner geworden. In het Haringvliet zelf verdwenen ook de zoutwaterplanten. Gelukkig is er inmiddels ook erkenning voor de schadelijke gevolgen van de afsluiting van het Haringvliet. Er wordt nu over gedacht om het getij deels terug laten keren, zoals ook bij de Stormvloedkering in de Oosterschelde mogelijk is gebleken.

Veranderingen in water- en bodemkwaliteit

Ook de waterkwaliteit van de rivieren werd steeds meer door de mens veranderd. In de eerste helft van de jaren '70 was de rivierwaterkwaliteit het slechtst. Doordat huishoudelijk water ongezuiverd in de rivier werd geloosd, bevatte het water zo weinig zuurstof dat vissen doodgingen. Sinds die tijd is de kwaliteit van het water sterk verbeterd. Toch zijn er nog steeds grote verschillen met de oorspronkelijke kwaliteit die het water had: in het water de Rijn en de Waal zit ongeveer 10x zoveel zout (natrium en chloride), 10 x zoveel nitraat en 2x zoveel sulfaat als in de oorspronkelijke situatie.
Ook in de chemische samenstelling van de bodem veranderde er veel. Veel uiterwaarden werden bemest, waardoor er meer fosfaat en stikstof in de bodem kan zitten.

Waar dit proefschrift over gaat

De veranderingen in de water- en bodemkwaliteit roepen de vraag op of de huidige uiterwaarden wel zo geschikt zijn om er nieuwe natuurgebieden in aan te leggen, vooral als er de wens ligt om bepaalde karakteristieke en kritische plantensoorten terug te laten keren of zich te laten vestigen. Deze vraag ligt ten grondslag aan dit proefschrift. We hebben deze vraag benaderd door naar biogeochemische processen in 'wetlands' ('natte natuurgebieden') in het rivierengebied te kijken. Biogeochemie is de wetenschap die zich bezighoudt met de chemische omzettingen ('chemie') in de bodem ('geo') door onder andere bacteriën en schimmels ('bio') en de effecten hiervan op de ecologie. Voor de vegetatie kunnen die effecten op twee gebieden liggen: op het voedselrijker worden van de bodem ('eutrofiëring') of giftigheid ('toxiciteit').

De toename van de voedselrijkdom is, in mijn ogen, het grootste probleem waar de Nederlandse natuur momenteel onder lijdt. Planten halen hun energie uit zonlicht, maar hebben wel allerlei bouwstoffen nodig om te groeien. Deze voedingsstoffen (meststoffen) halen ze uit de bodem, het water of de lucht. De

Spindotter in De Biesbosch.

voedingsstoffen waar het in dit onderzoek om draait, zijn stikstof (N), fosfor (P) en kalium (K). Verschillende soorten planten hebben verschillende manieren om met voedingsstoffen om te gaan. Er zijn langzame groeiers, die zuinig zijn met voedingsstoffen en aanpassingen hebben om ook als er weinig voedingsstoffen zijn nog te kunnen groeien. Daarnaast zijn er snelle groeiers, die meer verspillen en alleen kunnen groeien als er veel voedingsstoffen zijn. Door landbouw, verkeer en huishoudelijk afvalwater is de hoeveelheid voedingsstoffen in Nederlandse natuurgebieden sinds de jaren '50 sterk toegenomen. Het probleem hiervan is dat de snelle groeiers in veel gebieden de langzame groeiers overwoekeren, die dan vervolgens uit het gebied verdwijnen. Deze snelle groeiers zijn algemene planten, zoals brandnetels, terwijl de langzame groeiers vaak bijzondere soorten zijn die karakteristiek zijn voor een gebied.

Giftigheid klinkt als een negatief begrip, maar dat hoeft niet altijd zo te zijn. Er zijn stoffen die giftig zijn voor planten, maar die toch van nature in een gebied horen. De voor planten giftige stoffen die wij hier bestudeerd hebben, zijn sulfide (te herkennen aan de rotte-eierenlucht), chloride ('zout') en (gereduceerd) ijzer. Sulfide wordt gevormd als er sulfaat aanwezig is en dat zit momenteel twee keer zoveel in het rivierwater als vroeger. Ook chloride (zout) zit veel meer in het rivierwater. In dit proefschrift hebben we echter alleen gekeken naar chloride in het voormalige brakwatergetijdengebied. Door de afsluiting van de verbindingen met zee is daar juist veel minder zout aanwezig dan vroeger. Dit betekent dat nu vooral algemene planten groeien waar vroeger alleen planten voor konden komen die aangepast zijn aan zout.

IJzer is een belangrijk bestanddeel van de bodem. Het bijzondere van ijzer is echter dat het niet altijd onderdeel blijft van die bodem. Als er weinig zuurstof is, gaat ijzer in oplossing en kan het in het grondwater meestromen naar een ander gebied. Sommige gebieden zijn hierdoor erg rijk aan ijzer, terwijl andere juist bijna geen ijzer bevatten. Door sterk menselijk ingrijpen in de grondwaterstromen in Nederland wordt er in veel gebieden minder ijzer aangevoerd dan vroeger.

Nu is de vraag hoe biogeochemische processen ingrijpen op deze voedingsstoffen en giftige stoffen in de bodem. Hiervoor is het vooral van belang wat er gebeurt als er een overstroming plaatsvindt, of andersom, als een gebied droogvalt. Omzettingsprocessen in de natuur worden met name uitgevoerd door bacteriën en schimmels. Bacteriën gebruiken, net als mensen, organische stof (zoals resten van planten en dieren) om energie te krijgen. Hiervoor gebruiken de meeste, net als wij, zuurstof om deze organische stof te verbranden, waarbij CO_2 vrijkomt. Bij een overstroming of bij vernatting wordt er echter nagenoeg geen zuurstof meer naar de bodem toegevoerd. Er zijn echter andere bacteriën die geen zuurstof nodig hebben om organische stof te

verbranden. In plaats van zuurstof gebruiken zij dan nitraat, mangaan, geoxideerd ijzer of sulfaat. En als deze stoffen allemaal op zijn, zijn er nog steeds bacteriën die in staat zijn om energie te halen uit organische stof. Er ontstaat dan niet alleen CO_2 (kooldioxide), maar ook het gas methaan ('moerasgas', waar aardgas grotendeels uit bestaat).

De omzettingen die bacteriën uitvoeren in de bodem, grijpen op verschillende manieren in op voedselrijkdom en toxiciteit. Tijdens een overstroming zal de voedingsstof nitraat uit de bodem verdwijnen, maar de voedingsstof fosfaat komt juist vrij. Fosfaat zit sterk gebonden aan ijzer in de bodem, maar als bacteriën deze ijzeroxiden ('roest') omzetten naar gereduceerd ijzer, bindt er veel minder fosfaat aan. Dit fosfaat komt vervolgens vrij in het bodemwater en is dan makkelijk voor planten op te nemen. Gereduceerd ijzer en sulfide ontstaan tijdens de omzetting van geoxideerd ijzer in de bodem en sulfaat in het water. Het interessante is echter dat sulfide aan ijzer bindt. Als er dus ijzer aanwezig is als er sulfide ontstaat, wordt er ijzersulfide gevormd, waardoor zowel ijzer als sulfide niet meer giftig zijn. Hier komt echter bij dat, als dit gebeurt, fosfaat niet meer aan dit ijzer kan binden en hierdoor vrijkomt in het bodemwater.

Resultaten van het onderzoek

In dit onderzoek hebben we meestal gebruik gemaakt van plaggen: begroeide uitgestoken stukken bodem. Deze plaggen hebben we uit het veld meegenomen naar het laboratorium, de kas of een proefopstelling buiten om te bestuderen wat er gebeurt als bepaalde omstandigheden gecontroleerd veranderd werden, zoals de sulfaatconcentratie in het rivierwater of de getijdebeweging. Deze veranderingen hebben we onderzocht aan monsters die we van het bodemvocht, van de bodem en van het plantmateriaal namen.

Een experiment dat uitgevoerd werd bij verschillende temperaturen (hoofdstuk 2), bracht aan het licht dat omzettingsprocessen veel sneller verlopen tijdens een zomeroverstroming dan tijdens een winteroverstroming.

We hebben verschillende bodems met een verschillende bemestingsgeschiedenis laten overstromen (hoofdstuk 3). Hoeveel fosfaat er vrijkomt bij overstroming bleek niet direct gerelateerd aan de bemestingsgeschiedenis van een gebied: hier is ook ijzer in de bodem van belang. De verhouding tussen fosfaat en ijzer in de bodem, is hier uiteindelijk bepalend voor.

Verschillende bodems reageren verschillend op overstroming met sulfaatrijk water (hoofdstuk 4). Als er veel ijzer in de bodem zit, wat in de uiterwaarden vaak het geval is, zal het toxische sulfide niet snel vrijkomen vanwege de

binding aan ijzer. Bij weinig ijzer, zoals in veenbodems, kan dit wel gebeuren. Er kan extra fosfaat vrijkomen door de binding aan ijzer, maar in dit onderzoek was die hoeveelheid erg klein ten opzichte van de hoeveelheid die door vernatting (overstroming) vrijkwam. In dit onderzoek hebben we het effect van sulfaat ook uitgetest op een drijvende veenbodem ('kragge'). Dit drijvende veen, dat een beginstadium vormt in de verlanding van een plas, is erg zeldzaam en waardevol in de uiterwaarden. Onder invloed van sulfaat zonk de kragge. Kragges drijven (onder andere) op methaanbelletjes. Doordat er veel sulfaat was, kon er geen methaan meer gevormd worden. Eerst gebruiken bacteriën namelijk het sulfaat, omdat er minder energie vrijkomt bij de productie van methaan dan dat de oxidatie van organische stof met sulfaat oplevert.

Als er weer een getijdebeweging met invloed van zout ingesteld wordt in het Haringvliet, kan dit een positief effect hebben op planten die in een brakke omgeving horen (hoofdstuk 5). Zelfs bij lage zoutconcentraties moeten planten al energie stoppen in het onschadelijk maken van het giftige zout. Dat er in brak water meer sulfaat en chloride zit dan in zoet water, betekent niet dat er giftig sulfide vrijkomt of dat er extra fosfaat vrijkomt. Bovendien zorgt het verhogen van de getijdenbeweging ervoor dat er extra zuurstof de bodem in kan komen, waardoor fosfaat juist weer aan ijzer kan binden.

Er wordt vaak gedacht dat de Nederlandse uiterwaarden al zo voedselrijk zijn dat de aanvoer van extra voedingsstoffen geen effect meer heeft op de plantengroei. De vraag is dus of het erg is als er veel meststoffen in het rivierwater zitten. Om dit te achterhalen deden we een experiment met bodem en planten uit hooilanden waar de zeldzame Wilde kievitsbloem nog voorkomt (hoofdstuk 6). Het bleek dat stikstof, en in één van de hooilanden ook kalium, wel degelijk effect hebben op de plantengroei. Het bleek ook dat stikstof ervoor zorgt dat er meer grassen en minder andere soorten planten in het hooiland gaan groeien.

De Wilde kievitsbloem

Betekenis voor het beheer van rivieren

Uit het onderzoek blijkt dat de waterkwaliteit van rivieren een grote invloed kan hebben op de natuur waar het water mee in contact komt. Stikstof dat meegevoerd wordt met het rivierwater kan ervoor zorgen dat zeldzame vegetaties achteruitgaan. Hoewel dit misschien geen opzienbarend resultaat lijkt, is het toch van belang voor het huidige waterkwaliteitsbeleid, dat er vanuit gaat dat alleen fosfor belangrijk is. Daarnaast is niet alleen de waterkwaliteit, maar ook de bodemkwaliteit van belang. De gevolgen van overstroming of vernatting zijn afhankelijk van bodemkenmerken. Het is daarom belangrijk dat, als er gezocht wordt naar een nieuwe locatie om natte natuur aan te leggen of om water tijdelijk op te slaan, deze bodemkenmerken meegewogen worden in de keuze voor de locatie. Het versterkte broeikaseffect zal naar verwachting leiden tot een toename van zomeroverstromingen en een hogere temperatuur tijdens winteroverstromingen. Mede door het snellere verloop van biogeochemische processen bij deze toekomstige overstromingen, zullen de nadelige gevolgen ervan toenemen.

In een natuurlijk rivierenlandschap zijn er plekken met een hoge dynamiek dicht bij de rivier en plekken verder van de rivier die minder vaak in contact staan met de rivier en daardoor minder dynamiek kennen. Bij deze 'rustigere' plekken kun je denken aan oude, afgesloten riviermeanders en moerasbossen. In Nederland liggen deze plekken nu binnendijks en ze herbergen nog steeds bijzondere natuur. Hier kunnen zich nog laagveenmoerassen ontwikkelen en hier zijn bijvoorbeeld ook orchideeënrijke hooilanden in oude kleiwinputten te vinden. Hoewel deze natuurgebieden niet meer in contact staan met de rivieren, zouden ze niet hebben bestaan als de rivieren er niet geweest waren. Met het huidige systeem van dijken en uiterwaarden, zijn er geen mogelijkheden om dit soort plekken opnieuw te laten ontstaan. Daarom verdienen deze gebieden extra aandacht en bescherming.

De kijk op natuur in de uiterwaarden is drastisch veranderd nadat in 1987 een nieuw plan voor riviernatuur, Plan Ooievaar, werd gepresenteerd. Waar in andere natuurplannen altijd de focus op het behoud van bepaalde planten- en diersoorten lag, lag die in Plan Ooievaar op processen. Om ervoor te zorgen dat de uiterwaarden weer natuurlijker gingen functioneren, zoals ze vóór de menselijke beïnvloeding deden, moesten deze processen weer hersteld worden. Er moest weer meer contact komen tussen de uiterwaarden en de rivier, en grote grazers, zoals Konikpaarden, Galloways en Schotse Hooglanders, konden worden ingezet ter vervanging van wilde grazers die de overstromingsvlakten vroeger open hadden gehouden. Nagenoeg alle nieuwe natuurgebieden in de uiterwaarden zijn volgens dit principe ('natuurontwikkeling') ingericht. Het grote verschil tussen deze visie en de visie op natuur in andere landschappen

Galloway in natuurontwikkelingsgebied de Baarsemwaard bij Culemborg.

is dat de referentiesituatie anders is. Bij natuurontwikkeling wordt duidelijk gekozen voor een landschap zoals dat er vóór de menselijke beïnvloeding moet hebben uitgezien. In andere landschappen, zoals op heides en in laagveengebieden, wordt juist uitgegaan van de historische situatie die door menselijke benutting is ontstaan. In het beheer wordt dan aangesloten bij het vroegere gebruik van bijvoorbeeld maaien en plaggen. Dit type beheer heeft een grote soortenrijkdom opgeleverd. Ook in de uiterwaarden kunnen hooilanden voorkomen die voor het natuurbehoud in Europa van belang zijn. Ik denk dat het goed zou zijn als ook dit 'half-natuurlijke' beheer meer in nieuwe natuurgebieden in de uiterwaarden zou worden toegepast, naast gebieden waarin gekozen is voor natuurontwikkeling. Dit zou voor zowel de biodiversiteit als voor het behoud van cultuurhistorische waarden bevorderlijk zijn.

Dankwoord

Veel mensen denken dat het schrijven van een proefschrift eenzaam geploeter is. Gelukkig klopt dit beeld niet. In de afgelopen jaren hebben ontzettend veel mensen mij geholpen. Helaas kan ik niet iedereen bedanken, want dan zou dit dankwoord ongeveer even lang worden als de rest van het proefschrift. Een aantal mensen wil ik wel graag met name noemen.

Leon Lamers, ik had me geen betere begeleider voor kunnen stellen! Je maakte altijd uitgebreid tijd om mij te helpen, of het nu ging om het verbeteren van teksten, om ondersteuning bij veldwerk en experimenten of om gewoon een kletspraatje. Ik weet niet of ik zonder jouw begeleiding in staat was geweest om dit proefschrift op deze manier af te ronden (of eigenlijk weet ik dat wel…). Leon, nu is het af en nu is het ook echt eindelijk tijd om naar huis te gaan!

Jan Roelofs, graag wil ik je bedanken voor de enthousiaste ideeën die je elke keer hebt aangedragen. Bedankt dat je me de rivier de Shannon in Ierland hebt laten zien (zie foto hiernaast). Niet alleen de andere geomorfologie, maar ook het andere beheer dat in de Shannon Callows plaatsvindt, waren voor mij een eye-opener. Bedankt voor alle congressen die ik in deze jaren, met en zonder jou, heb mogen bijwonen!

Martijn Antheunisse en Marzia Miletto waren mijn mede-AIO's in dit project vanuit de Universiteit Utrecht en NIOO-KNAW in Nieuwersluis. Ik wil jullie beide heel erg bedanken voor de goede samenwerking en alle gezelligheid (Marzia, ik weet dat je dit kunt lezen in het Nederlands)! Ik heb erg goede herinneringen aan ons veldwerk (met waadpakken aan bij laag tij in Heinenoord …), het gezamenlijke Haringvlietexperiment en niet te vergeten onze geweldige excursie naar de rivieren Oder, Bug en Narew in Polen. Jos Verhoeven, Riks Laanbroek en Paul Bodelier, consortiumleden in het project, ik vond onze gezamenlijke projectvergaderingen elke keer weer inspirerend.

Veel van het werk in dit proefschrift is uitgevoerd door studenten die een master- of bachelorstage bij mij hebben gelopen. In volgorde-van-opkomst waren dit Etienne van Boxsel, Denise Buijk, Annemarie Lammers, Erika van Daalen, Lisa Kuijpers en Sjaak Weenink. Ik wil jullie allemaal heel erg bedanken voor het onmisbare werk dat jullie hebben verzet! Ik hoop dat jullie stages voor jullie even leerzaam zijn geweest voor jullie als dat ze voor mij waren.

Veel lab- en veldassistentie werd verleend door Germa Verheggen, Roy Peters en Martin Versteeg. Germa en Roy, heel erg bedankt voor het verwerken van honderden monsters (nu kan eindelijk de koelcel weer leeggemaakt worden)! Martin, bedankt voor alle hulp, inventiviteit en gezelligheid in het veld! In het Gemeenschappelijk Instrumentarium heb ik veel hulp gehad van Jelle Eygensteyn, Liesbeth Pearson en Rien van der Gaag. Jelle, ik heb veel van je geleerd over analytische chemie in al die dagen dat ik bij jou achter de auto-analysers heb gestaan! De experimenten die ik in hoofdstuk 5 en 6 heb beschreven, zijn in en om de kassen uitgevoerd. Graag wil ik alle medewerkers van de kassen, met name Gerard van de Weerden en Yvette Evers, bedanken voor alle goede zorgen.

Vanuit NWO werd het onderzoeksproject begeleid door Helenius Rogaar, Hugo Coops, Peter Doelman, Gerard Muyzer, Jan Streefkerk, Jos Vink en Jaap van der Waarde. De halfjaarlijkse vergaderingen vormden elke keer een goed moment om terug en vooruit te kijken in het project en de deelprojecten weer op elkaar af te stemmen.

Mijn dank gaat ook uit naar alle natuurbeherende organisaties en particulieren die ons tijdens het project toestemming hebben verleend voor het nemen van monsters, plaggen en vegetatie-opnamen op hun terrein. In het bijzonder wil ik hier Jeroen Breedenbeek van Staatsbosbeheer bedanken, die toestemming gaf om de zeldzame en kwetsbare Kievitsbloemhooilanden in zijn regio te onderzoeken. Ik hoop dat dit proefschrift een bijdrage kan leveren aan een beter beheer van natuurgebieden in het rivierengebied.

Jan Klerkx (Bèta Vertalingen) corrigeerde het Engels van de hoofdstukken 2 tot en met 6. Hoewel er aan het laatste hoofdstuk nog ongeveer evenveel verbeterd moest worden als aan het eerste, heb ik toch het gevoel dat ik er veel van geleerd heb. Hartelijk dank daarvoor!

Niets is zo belangrijk als goede collega's. Naast de mensen die ik hierboven al bedankt heb, wil ik ook de volgende mensen bedanken voor de goede sfeer en samenwerking: Marlies van der Welle, Jeroen Geurts en Artur Banach, bedankt voor de gezelligheid in de periode dat wij 'kamergenoten' waren! Marlies en Jeroen, ik vind het fijn dat jullie mijn paranimfen willen zijn. Verder dank aan

Dries Boxman, Philippine Vergeer, Leon van den Berg, Tjisse van der Heide, Peter Spierenburg, Monique van Kempen, Maaike de Graaf, José Broekmans, José Adema, Maria van Kuppenveld en alle andere (oud-)medewerkers van milieubiologie. Van Onderzoekscentrum B-Ware (en de periode ervoor) wil ik graag Fons Smolders, Hilde Tomassen, Esther Lucassen, Emiel Brouwer, Bert Hennipman en Rick Kuiperij (en alle anderen!) bedanken voor de goede samenwerking. Iedereen van Moleculaire Ecologie, Experimentele Planteneco-logie en Dierecologie, bedankt voor alle gezellige gezamenlijke momenten! Mijn huidige collega's van Alterra wil ik bedanken voor de interesse die zij voor mijn schrijfwerk hebben getoond.

Mamma en Iris, het was leuk om tegelijk met jullie aan een promotie-onderzoek aan dezelfde universiteit te werken. De lunchwandelingen in de botanische tuin waren net zo als vroeger-toen-we-klein-waren. Doordat jullie ook bezig waren met een promotiestudie, hield ik gelukkig het gevoel dat promoveren iets heel gewoons is. Pappa, alhoewel je nu de enige uit het gezin bent die niet gepromoveerd is (het kan altijd nog!), denk ik toch dat jij degene bent die het meest van dit proefschrift snapt. Jij hebt mij de basis van de scheikunde bijgebracht.

Lieve Rob, heel erg bedankt voor alle steun in de afgelopen jaren! Het was erg fijn om iemand te hebben die begrijpt wat je aan het doen bent, die teksten doorleest, af en toe kritische vragen bij experimenten stelt en voor je kookt als je laat thuiskomt. Je hebt het proefschrift prachtig vormgegeven! Ik hoop dat we nu weer meer tijd krijgen om samen lekker naar buiten te gaan!

Curriculum vitae

Roos (Rozemarijn) Loeb werd op 24 april 1977 geboren in Renkum. Zij deed VWO aan het Wagenings Lyceum (dat later 'het Wagenings' werd) in Wageningen, waar zij in 1995 eindexamen deed. Daarna studeerde zij milieuhygiëne aan de Landbouwuniversiteit Wageningen (later Wageningen Universiteit). Haar interesse ontwikkelde ze vooral in de richting van chemische processen in de bodem en de interacties ervan met planten. Zij specialiseerde zich daarom in bodemhygiëne en natuurbeheer. Haar eerste afstudeervak ging over veranderingen in fosforvormen tijdens successie in natte duinvalleien op Terschelling, dat ze bij de vakgroep Terrestrische Oecologie en Natuurbeheer en de vakgroep Bodemkunde en Plantevoeding uitvoerde. Haar tweede afstudeervak ging over een manier om de toxiciteit van nikkel in de bodem voor planten te bepalen, ook bij de vakgroep Bodemkunde en Plantevoeding. Aan het eind van haar studie liep zij stage bij Kiwa Water Research in Nieuwegein. Hier hielp zij bij de implementatie van een indicatormodule in het programma Synbiosys. Haar doctoraal sloot zij in 2001 cum laude af.

Haar liefde voor de natuur deed Roos op bij de Jeugdbond voor Natuur- en Milieustudie. Tijdens haar middelbareschooltijd en haar studie was ze eerst actief in afdeling Ede-Wageningen, en daarna in de plantenwerkgroep en op landelijk bestuursniveau. In 1998 was zij secretaris in het Dagelijks Bestuur van de JNM.

Na haar studie werkte zij bij Kiwa Water Research in opdracht van Brabant Water aan een ecohydrologische quickscan van natuurgebieden in Noord-Brabant. In 2002 begon zij aan haar promotie-onderzoek aan de Katholieke Universiteit Nijmegen (thans Radboud Universiteit Nijmegen) bij de afdeling Aquatische Oecologie en Milieubiologie. Het promotie-onderzoek maakte deel uit van het project 'Biogeochemical constraints for sustainable development of floodplains in riverine regions' in het programma 'Tripartite approach to soil system processes', gefinancierd door NWO. Sinds mei 2007 werkt Roos als onderzoeker aquatische ecologie bij Alterra, Wageningen UR.

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